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(54) Title: PHASE SHIFT COLLOIDS AS ULTRASOUND CONTRAST AGENTS

(57) Abstract

Agents for enhancing the contrast in a diagnostic ultrasound procedure comprise colloidal dispersions of the liquid-in-liquid type, i.e., emulsions or microemulsions, in which the dispersed liquid phase is a liquid having a boiling point below the temperature of the animal to be imaged and which therefore undergoes a phase change from a dispersed liquid to a highly echogenic dispersed gaseous foam or kugelschaum following administration to the animal. The liquid state of the dispersed phase allows one to manufacture extremely stable, pharmaceutically acceptable emulsions with particle sizes typically below 1000 nm. The gaseous state at body temperature yields highly echogenic microbubbles, typically below 10,000 nm in diameter, which are effective as ultrasound contrast agents. Intravenous, intraarterial, oral, intraperitoneal, and intrauterine dosage forms, methods of administration, and imaging techniques are described.

PHASE SHIFT COLLOIDS AS ULTRASOUND CONTRAST AGENTS

RELATED APPLICATIONS

This application is a continuation-in-part of
pending application S.N. 08/148,284 filed November 8,
5   1993 which is a continuation-in-part of pending
application S.N. 08/008,172, filed January 25, 1993.

FIELD OF THE INVENTION

The present invention is directed to contrast
agents for biomedical use comprising aqueous
10   colloidal dispersions. More specifically, the
present invention is directed to liquid in liquid
emulsions in which the dispersed liquid undergoes a
temperature or pressure activated phase shift from a
dispersed liquid to a dispersed gaseous form which is
15   efficient in reflecting ultrasound energy in a manner
which is diagnostically useful.

BACKGROUND OF THE INVENTION

Various contrast agents for use with diagnostic
ultrasound, including echocardiography, have been
20   described. A review of the subject is found in Ophir
and Parker, Ultrasound in Med. & Biol. (1989),
15:319-333. The acoustic backscatter arising from
these agents, the property typically associated with
the contrast effect, can be attributed to unique
25   properties which they possess as solids, liquids or
gases. While solids and liquids reflect sound to a
similar degree, gases are known to be more efficient
and are the preferred media for the development of
ultrasound contrast agents.
30   Known liquid agents for ultrasound include
emulsions and aqueous solutions. About these the
authors of the above review stated, "the idea of
using liquid emulsions of certain lipids in aqueous

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vehicles was tested by Fink et al. (1985).
Unfortunately, no enhancement of backscatter was
observable in these experiments."

5   Known solid agents include collagen
microspheres. However, the poor acoustic backscatter
of the solid-liquid interface prevents their wide
spread use.

Known gaseous agents include microbubbles
stabilized by the addition of various amphiphilic
10   materials to the aqueous media, by materials that
increase viscosity, and gaseous precursors, either as
solid particles or liposomes. However, the liposomes
can only contain water soluble gases and are thus
limited in the stability of the microbubbles they can
15   form, since one of the characteristic physical
properties of many of the chemicals which form
especially stable microbubbles is immiscibility in
water. The solid particles must be reconstituted
immediately before use, requiring extensive
20   preparation, and must be used quickly, since the
microbubbles disappear soon after the particles have
completely dissolved. My own prior U.S. patent
application S.N. 07/761,311 is directed to methods of
determining the relative usefulness of gases as
25   ultrasound contrast agents, and identifies
particularly useful gases for that purpose.

One study has been identified which used the
injection of a liquid which boils at a temperature
below the boiling point of the organism under study
30   to enhance the ultrasound Doppler signal (Ziskin MC,
Bonakdarpour A, Weinstein DP, Lynch PR: Contrast
Agents For Diagnostic Ultrasound. Investigative
Radiology 7:500-505, 1972). In this study a number
of solutions or liquids were injected intraarterially
35   into dogs and the Doppler signal detected five cm
below the injection site. This study reported that,

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"ether, which produced the greatest contrast effect
of any agent that we tried, is a liquid which boils
vigorously at body temperature and therefore acts as
a very active source of bubbles." The report further

5   stated that "ether, however, is a toxic substance
when injected in large amounts. Injections of 20 mL
proved fatal in our experiments." This paper does
not discuss methods of stabilizing any materials
suitable for later use as ultrasound agents. Non-

10   colloidal ether is too toxic for intravenous
administration, where the greatest need for a useful
contrast agent exists.

The biocompatability of emulsions which include
fluorocarbons is a serious safety concern. For

15   example, Clark et al. (Clark LC, Becattini F, Kaplan
S: Can fluorocarbon emulsions be used as artificial
blood? Triangle 11:115-122, 1972) state, in speaking
about the choice of fluorocarbon, "their vapor
pressures range from zero to about 640 torr. Those

20   with vapor pressures over 400 torr, of course, cannot
be used because they would boil when infused in the
blood stream." Later in the same article they state,
"If a fluorocarbon with a vapor pressure of over 50
torr is given intravenously, death results in a few

25   hours, and when the chest is opened, the lungs do not
collapse." The same author, L.C. Clark, reports a
similar conclusion exactly twenty years later, "If
practical methods cannot be found to prevent or
counteract HNCL (hyperinflated non-collapsible

30   lungs), and if HNCL occurs in other species, then
only fluorocarbons boiling above 150°C can be
considered safe," Clark CL, Hoffmann RE, Davis SL:
Response of the rabbit lung as a criterion of safety
for fluorocarbon breathing and blood substitutes,

35   Biomat., Art. Cells & Immob. Biotech., 20:1085-1099,
1992.

The stability of liquid-liquid emulsions presents another problem. A body of knowledge surrounds the stability of emulsions and the ability to predict stability from solubility; this theory is called the Ostwald ripening theory (Kabalnov AS, Shchukin ED; Ostwald Ripening Theory: Applications To Fluorocarbon Emulsion Stability, Advances in Colloid and Interface Science, 38:69-97, 1992). This paper states, simply, that the more soluble is the dispersed phase liquid of an emulsion in the continuous phase, the less stable is the emulsion. These same authors tested the stability of a dodecafluoropentane emulsion at 25°C (Kabalnov AS, Makarov KN, Shcherbakova OV: Solubility of fluorocarbons in water as a key parameter determining fluorocarbon emulsion stability. J Fluorine Chemistry 50:271-284, 1990). They determined that their emulsion had an Ostwald ripening rate of 1.4×10^{-18} cm^3/s. Converting this rate constant into useful terms shows that Kabalnow et al's dodecafluoropentane emulsion, which had an initial size of 211 nm, would experience a particle mean diameter growth rate of 11 nm/sec or 660 nm/minute. At this rate of particle growth, such an emulsion would have a shelf life of less than a minute, and therefore be unworkable as a commercial product.

Thus, there is a need for an effective ultrasound contrast composition with extended shelf life, which is relatively easy to manufacture, and which is biocompatible and convenient to use.

SUMMARY OF THE INVENTION

In order to meet these needs, the present invention is directed to stable colloidal dispersions of the liquid-in-liquid type. The colloids are composed of a liquid dispersed phase which has a

boiling point below the body temperature of the
organism on which an ultrasound contrast study is
desired, typically about 37-40°C. These emulsions
are preferably composed of a dispersed phase liquid
5   which has a boiling point between -20 and 37°C.
       Preferably the liquid dispersed phase is
selected from the group of chemicals consisting of
aliphatic hydrocarbons, organic halides or ethers, or
combinations thereof, which have six or fewer carbon
10   atoms and an upper limit of molecular weight of about
300. Among organic halides, the fluorine-containing
chemicals are preferred, since they form stable
emulsions and are relatively non-toxic. Especially
preferred are n-pentane, isopentane, neopentane,
15   cyclopentane, butane, cyclobutane, decafluorobutane,
dodecafluoropentane, dodecafluoroneopentane,
perfluorocyclopentane and mixtures thereof.
Preferably, the colloidal dispersion contains the
dispersed phase at a concentration of 0.05 to 5.0%
20   w/v. Optimally, the concentration range is 0.5 to
3.5% w/v.
       The colloidal dispersion can be stabilized by
the addition of various amphiphilic materials,
including anionic, nonionic, cationic, and
25   zwitterionic surfactants, which typically lower the
interfacial tension between the dispersed liquid and
water to below 26 dynes/cm. Optimally, these
materials are nonionic, synthetic surfactant
mixtures, containing a fluorine-containing
30   surfactant, such as the Zonyl brand series and a
polyoxypropylene-polyoxyethylene glycol nonionic
block copolymer.
       The liquid continuous phase of the colloidal
dispersion comprises an aqueous medium. This medium
35   can contain various additives to assist in
stabilizing the dispersed phase or in rendering the

formulation biocompatible. Acceptable additives
include acidifying agents, alkalizing agents,
antimicrobial preservatives, antioxidants, buffering
agents, chelating agents, suspending and/or
5   viscosity-increasing agents, including triodobenzene
derivatives, such as iohexol or iopamidol, and
tonicity agents. Preferably, agents to control the
pH, tonicity, and increase viscosity are included.
Optimally, a tonicity of at least 250 mOsm is
10.   achieved with an agent which also increases
viscosity, such as sorbitol or sucrose.

The colloidal dispersions are typically formed
by comminuting a suspension of the dispersed phase in
the continuous phase by the application of
15   mechanical, manual, or acoustic energy. Condensation
of the dispersed phase into the continuous phase is
also acceptable. The preferred mode is to use high-
pressure comminution.

DETAILED DESCRIPTION OF THE INVENTION

20   The invention relates to agents that enhance the
contrast in an ultrasound image generated for use in
medical and veterinary diagnosis. These agents are
comprised of biocompatible colloidal dispersions in
which the dispersed phase is a liquid under the
25   conditions of the manufacturing process and which
undergoes a phase shift to become a dispersed gas or
kugelschaum at or about the time of administration to
the organism under study.

In order to provide a clear and consistent
30   understanding of the present invention and claims,
including the scope given to such terms, the
following definitions relating to the invention are
provided:

Colloidal Dispersion: A system having at least
35   one substance as a liquid or gas (the dispersed

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phase) which is immiscible and finely divided and distributed evenly throughout at least one second substance which forms the dispersion medium or continuous liquid phase.

5   Biocompatible: Capable of performing functions within or upon a living organism in an acceptable manner, without undue toxicity or physiological or pharmacological effects.

           Liquid: The state of matter in which a substance
10   or substances exhibit(s) a characteristic readiness to flow, little or no tendency to disperse, and relatively high incompressibility.

           Gas: The state of matter of a substance or substances which is distinguished from the solid or
15   liquid states by very low density and viscosity, relatively great expansion and contraction with changes in temperature and pressure, and the spontaneous tendency to become distributed uniformly throughout any container.

20   Phase Shift: A change of state between liquid and gas due to changes in temperature and/or pressure.

           Kugelschaum: One of the two forms of foams in the classification of Manegold (Manegold, E. "Schaum,
25   Strassenbau, Chemie und technik." Heidelberg, 1953, which is incorporated herein by reference). Specifically, the kugelschaum or spherical foam, consists of widely separated spherical bubbles and is distinct from the polyederschaum or polyhedral foams,
30   which consist of bubbles that are nearly polyhedral in shape, having narrow lamellar films of very low curvature separating the dispersed phase.

           Low Boiling Liquid: A liquid with a boiling point, under standard pressure conditions, below 40°
35   C.   Low boiling liquids useful in the invention include, but are not limited to, hydrocarbons,

organic halides, and ethers, where, in any case, the
molecule has 6 carbon atoms or less.

     Aliphatic Hydrocarbons: The group of alkane,
alkene, alkyne, cycloalkane, and cycloalkene organic

5   compounds. Of these, only compounds having boiling
points below about 40°C (such as those having six or
fewer carbon atoms) and which are thus capable of
undergoing a liquid to gas phase transition after
administration to a subject from part of this

10   invention. Aliphatic hydrocarbons useful in the
invention include, but are not limited to, those
selected from the chemical group: Isobutane;
Isobutylene; 1-Butene; 1,3-Butadiene; n-Butane; 2-
Butene (trans); 2-Butene (cis); Vinyl acetylene; 1-

15   Butyne; Neopentane; Butadiyne; 1,2-Butadiene;
Cyclobutane; 1-Butene, 3-methyl; Cyclopropane, 1,1-
dimethyl; 1,3-Dioxolane-2-one, 4-methyl; 3-Butene-2-
one, 4-phenyl (trans); 1,5-Heptadiyne; 1,4-
Pentadiene; 2-Butyne; Butane, 2-methyl; Cyclopropane,

20   1,2-dimethyl (trans, dl); 1-Butyne, 3-methyl; 1-
Pentene; 1-Butene, 2-methyl; 1,3-Butadiene, 2-methyl;
1-Butene-3-yne, 2-methyl; Isoprene; Cyclopropane,
ethyl; n-Pentane; Cyclobutane, methyl; 2-Pentene
(trans); 2-Pentene (cis); Cyclopropane, 1,2-dimethyl

25   (cis); and 1-Nonene-3-yne.

     Organic Halides: The group of compounds
containing at least one carbon or sulfur atom and at
least one halogen atom, i.e., chlorine, bromine,
fluorine, or iodine. Of these, only the members of

30   the group having boiling points below about 40°C
(such as those with six or fewer carbon atoms) which
are capable of undergoing a phase transition upon
administration to an organism with a body temperature
of up to 40°C form part of the invention. Examples

35   of such organic halides include: Methane,
tetrafluoro; Methane, chlorotrifluoro; Ethane,

hexafluoro; Ethane, perfluoro; Methane, fluoro; Ethylene, tetrafluoro; Sulfur hexafluoride; Methane, bromotrifluoro; Methane, difluoro; and like compounds.

Ethers: The class of organic compounds in which two hydrocarbon groups or derivatives thereof are linked by an oxygen atom. For the purposes of the present invention the following are examples of some, but not necessarily all, ethers which can be used: methyl ether, ethyl methyl ether, methyl vinyl ether, methyl isopropyl ether, 1,2-epoxypropyl ether, diethyl ether, ethyl vinyl ether, and vinyl ether.

Fluorine-Containing Compounds: A compound containing at least one fluorine atom. Some useful fluorine-containing compounds are listed above as organic halides. See also the examples below.

The colloidal dispersions of the invention can be emulsions or microemulsions.

Emulsion: A colloidal dispersion of one immiscible liquid dispersed in another liquid in the form of droplets, whose diameter, in general, are between 100 and 3000 nm and which is typically optically opaque, unless the dispersed and continuous phases are refractive index matched. Such systems possess a limited stability, generally defined by the application or relevant reference system, which may be enhanced by the addition of amphiphilic materials or viscosity enhancers.

Microemulsion: A stable liquid monophasic and optically isotropic colloidal dispersion of water and water-immiscible liquids stabilized by amphiphilic materials in which the dispersions have appreciable light scattering properties (meaning they can appear optically clear or milky but are reddish or yellowish if observed by transmitted light) and the diameters

of the particles are, in general, between 5 and
approximately 140 nm.

In a preferred embodiment of the present
invention, the colloidal dispersion contains one or
more amphiphilic materials to improve the stability
of the formulation.

Amphiphilic Material: A substance which is
strongly adsorbed at an interface and which normally
produces a dramatic reduction in the interfacial
tension with small changes in the bulk phase
concentration. Examples include synthetic
surfactants, naturally occurring materials such as
biocompatible proteins, lipids, sterols, alginates,
cellulose derivatives, and finely divided organic or
inorganic particulate solids.

Organic Particulate Solids: include sugars,
proteins, amino acids, lipids, nucleic acids, and
others.

Inorganic Particulate Solids: include aluminas,
carbonates, bicarbonates, silicates,
aluminasilicates, phosphates, and others.

Interface: The region or boundary of the
physical world that lies between two distinct and
identifiable phases of matter, herein limited to
liquid-liquid, liquid-solid, solid-gas, and liquid-
gas.

Interfacial Tension: The force per length which
exists at the interface between two distinct and
identifiable phases of matter.

Stability: The time lapse from initial
preparation and packaging during which a colloidal
dispersion continues to fulfill all chemical and
physical specifications with respect to identity,
strength, quality, and purity which have been
established according to the principles of Good

Manufacturing Practice, as set forth by appropriate
governmental regulatory bodies.

Surfactants: The group of amphiphilic materials
which are manufactured by chemical processes or

5   purified from natural sources or processes. These
can be anionic, cationic, nonionic, and zwitterionic,
as are well known in the art. Such materials are
described in Emulsions: Theory and Practice, Paul
Becher, Robert E. Krieger Publishing, Malabar,

10   Florida, 1965 which is incorporated by reference
herein.

The continuous phase of the colloidal dispersion
of the present invention is an aqueous medium.

Aqueous Medium: A water-containing liquid which

15   can contain pharmaceutically acceptable additives
such as acidifying agents, alkalizing agents,
antimicrobial preservatives, antioxidants, buffering
agents, chelating agents, complexing agents,
solubilizing agents, humectants, solvents, suspending

20   and/or viscosity-increasing agents, tonicity agents,
wetting agents or other biocompatible materials. A
tabulation of ingredients listed by the above
categories, can be found in the U.S. Pharmacopeia
National Formulary, 1990, pp. 1857-1859, which is

25   incorporated herein by reference.

A preferred embodiment of the present invention
includes the use of at least one amphiphilic material
from the groups consisting of biocompatible proteins,
fluorine-containing surfactants, polyoxypropylene-

30   polyoxyethylene glycol nonionic block copolymers, and
surfactants.

Polyoxypropylene-Polyoxyethylene Glycol Nonionic
Block Copolymers: The surfactants which are available
from BASF Performance Chemicals, Parsippany, New

35   Jersey under the trade name Pluronic and which
consists of the group of surfactants designated by

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the CTFA name of poloxamer 108, 188, 217, 237, 238,
288, 338, 407, 101, 105, 122, 123, 124, 181, 182,
183, 184, 212, 231, 282, 331, 401, 402, 185, 215,
234, 235, 284, 333, 334, 335, and 403.

5   Fluorine-Containing Surfactant: A surfactant
containing one or more fluorine molecules. Some but
not necessarily all fluorine containing surfactants,
useful in this invention can be selected from the
group consisting of: telomer B containing fluorinated
10   surfactants available from Du Pont, Wilmington, DE
under the Trade name of Zonyl (including Zonyl FSA,
FSP, FSE, UR, FSJ, FSN, FSO, FSC, FSK, and TBS),
the fluorochemical surfactants from 3M Industrial
Chemical Products Division, St. Paul, MN under the
15   trade name of Fluorad (including FC-95, FC-98,
FC-143, FC-170C, FC-171, FC-430, FC-99, FC-100,
FC-120, FC-129, FC-135, FC-431, FC-740), the
perfluoroalkylpoly(oxyethylene) surfactants described
by Mathis et al. (J Am Chem Soc 106, 6162-6171
20   (1984), incorporated herein by reference), the
fluoroalkylthio-etherpoly(oxyethylene) surfactants
described by Serratrice et al. (J Chim Phys 87,
1969-1980 (1990), incorporated herein by reference),
the perfluoroalkylated polyhydroxylated surfactants
25   of Zarif et al. (J Am Oil Chem Soc 66, 1515-1523
(1989), incorporated herein by reference), the
fluorosurfactants available from Atochem North
America, Philadelphia, PA under the trade name of
Forafac.

30   Biocompatible Proteins: The group of proteins,
regardless of source and whether obtained by
extraction of animal, plant, or microbiological
tissue or obtained from recombinant biotechnology,
which is capable of performing its function of
35   stabilizing the colloidal dispersions of the instant
invention in an acceptable manner, without undue

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toxicity or physiological or pharmacological effects. Some acceptable biocompatible proteins can be selected from the group consisting of albumin, alpha-1-antitrypsin, alpha fetoprotein, aminotransferases,

5   amylase, C-reactive protein, carcinoembryonic antigen, ceruloplasmin, complement, creatine phosphokinase, ferritin, fibrinogen, fibrin, transpeptidase, gastrin, serum globulins, hemoglobin, myoglobin, immunoglobulins, lactate dehydrogenase,

10   lipase, lipoproteins, acid phosphatase, alkaline phosphatase, alpha-1-serum protein fraction, alpha-2 serum protein fraction, beta protein fraction, gamma protein fraction, gamma-glutamyl transferase, and other proteins.

15   A preferred process for manufacturing the colloidal dispersions of this disclosure is comminution. An alternative process for manufacturing is condensation.

            Comminution: The process of forming a colloidal

20   dispersion by mixing the liquid dispersed and continuous phases together and then causing a decrease in size of the particles of the dispersed phase from large particles to the size required, using mechanical energy generated by mixing manually,

25   mechanically, or by the action of ultrasound. Appropriate mixing can be achieved in a Microfluidic's Model 110 Microfluidizer apparatus, as described in U.S. Pat. No. 4,533,254, incorporated herein by reference. An acceptable alternative is

30   the Rannie High Pressure Laboratory Homogeniser, Model Mini-Lab, type 8.30H, or equivalent.

            Condensation: The process of forming a colloidal dispersion by starting with the dispersed phase as a gas, placing it in contact with the liquid continuous

35   phase and then causing an increase in size of the particles of the dispersed phase from a molecular

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ensemble to the size required, generally by inducing
a phase change of the dispersed gas to a liquid by
the action of changes in the system temperature,
pressure, or both.

5   The invention will be better understood by way
of the following examples:

EXAMPLE 1
          The criticality that the low boiling liquid be
present as a finely divided dispersion rather than as
10   a neat liquid, as was described by Ziskin et al.
(referenced above) was determined by measuring the
acoustic backscatter of the two states.
          Two solutions were prepared to simulate the
administration to an organism of either a colloidal
15   dispersion of a low boiling liquid or the liquid
neat. These were scanned at 5.0 MHz with a Hewlett
Packard Model 77020 ultrasound scanner and the images
obtained recorded on Sony ES VHS tape. The analog
images from the tape were then converted to a digital
20   form using the software package Global Lab Image
Software (Data Translation, Marlboro, MA). The gray
scale intensity within a 4900 pixel (70 x 70 pixel-
sized) region-of-interest was then measured before
and after the injection of the colloidal dispersion
25   of Example 19 or a quantity of neat
dodecafluoropentane into a 1000 mL water beaker
equilibrated at 37°C.
          The measurements were performed on a gray scale
of 2 to 254. The image intensity before injection of
30   a 0.1 mL aliquot of the emulsion of Example 19 below
(containing 3.4 micromoles of dodecafluoropentane)
was 4.27. The injection of 0.1 mL of this emulsion
produced a change of intensity to 236 five seconds
post-injection and 182 fifty-two seconds post-
35   injection.

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The same experiment was performed with a 0.2 mL
injection of neat dodecafluoropentane. This
corresponds to 1111 micromoles of
dodecafluoropentane, over 300-times the quantity in

5   the experiment above. The image intensity before
injection was 4.9; this increased to 7.7 five seconds
post-injection and 5.0 fifty-two seconds post-
injection.

A comparison of these two experiments

10   (intensity/quantity) indicates that the colloidal
dispersion is 27,000-times more effective at
scattering the ultrasound beam than simply an
administration of a liquid which also undergoes a
liquid-to-gas phase transition.

15   EXAMPLE 2
The selection of an appropriate chemical for the
liquid dispersed phase is governed, in part, by the
body temperature of the organism to be studied by
ultrasound. For example, since the body temperature

20   of man is 37°C, liquids which undergo a liquid to gas
phase transition, i.e., boil, at or below 37°C are
especially useful in the colloidal dispersions of the
invention. In a similar manner, the following table
can be used as guidance in selecting the liquid

25   dispersed phase, depending on which organism is to be
studied:

ORGANISM	RECTAL TEMPERATURE (degrees Fahrenheit)
Swine (Sus scrofa)	101.5-102.5
Sheep (Ovis sp.)	101-103
Rabbit (Oryctolaqus cuniculus)	102-103.5
Rat (Tattus morvegicus)	99.5-100.6
Monkey (Macaca mulatta)	101-102
Mouse (Mus musculus)	98-101
Goat (Capra hircus)	101-103
Guinea pig (Cavia porcellus)	102-104
Hamster (Mesocricetus sp.)	101-103
Man (Homo sapiens)	98.6-100.4

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Horse (Equus sp.)	101-102.5
Dog (Canin familiaris)	101-102
Baboon (Papio)	98-100
Cat (Felis catus)	101-102
Cattle (Bos taurus)	101.5-102.5
Chimpanzee (Pan)	96-100

EXAMPLE 3

A colloidal dispersion was formed by comminuting, using the method and criteria of Example 45 below, an organic halide.

Specifically, a 100 mL quantity of a formulation was created containing: poloxamer 488, 2.5% v/v; fluorine-containing surfactant Zonyl FSN 2.5% v/v; sodium perfluorooctanoate, pH 7.0, 0.1% w/v; sodium chloride, 0.9%, w/v; and dodecafluoropentane, 2.0%, v/v. After low shear mixing, these were comminuted in the Microfluidizer model 110Y at 4°C for eight passes. The milky emulsion was aliquoted into serum vials and sealed.

Within 72 hours, the particle size and size distribution was determined at 19°C using the Nicomp model 370 (Nicomp Particle Sizing, Santa Barbara, CA). The mean diameter of the Gaussian analysis of the emulsion was 90.1 nm (number weighted) with a standard deviation of 48%. The volume weighted mean diameter was 316 nm.

EXAMPLE 4

The particle size and size distribution were determined at various steps or under different conditions during the formulation of an emulsion.

A 20 mL quantity of an emulsion was formulated, containing sodium perfluorooctanoate, pH 7.2, 2.5%, w/v, and dodecafluoropentane, 2%, w/v. These ingredients were added to water and the suspension cooled to 4°C. The Emulsiflex-1,000 (Avestin, Inc.,

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Ottawa, Canada) was used to "pre-mix" the solution
before final comminution.

Following 20 passes of the solution between two
10 mL syringes, the white, milky suspension was
placed in the Nicomp 370 to determine particle size.
This pre-mix suspension had a mean particle size
(number weighted) of 452 hm and (Volume weighted) of
2398 nm.

The final emulsion was then formed by
comminution through eight passes with the
Emulsiflex-1,000 (Avestin, Inc., Ottawa, Canada)
operating manually at a pressure of up to 7 MPa. The
emulsion particles were much smaller, with a number-
weighted mean diameter of 201 nm and a volume
weighted mean diameter of 434 nm.

Aseptic filling of the material was achieved by
passing the material through a 0.45 micron sterile
filter (Gelman Acrodisc, Ann Arbor, MI). The final,
sterile colloidal dispersion had a number weighted
mean diameter of 160 nm.

EXAMPLE 5
The mean particle size measurement of an
emulsion immediately after comminution is a useful
test of the ultimate stability of the formulation.
The following emulsions illustrate this point:

A 2%, v/v, dodecafluoropentane emulsion was
formulated containing 2% Pluronic P-123 and 2.6%
Zonyl FSO, according to the method of Example 19
below. The mean particle diameter was 151 nm, with a
35% standard deviation. This emulsion was stable for
at least six weeks, as judged by physical appearance
and particle size.

To the same formulation was added 0.25% sodium
perfluorooctonate. Although it was speculated this
might further stabilize the formulation because this

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addition reduces interfacial tension, the high
anionic charge density this surfactant could generate
at the emulsion interface may actually prevent
production of small particles. In fact, the
5   immediate particle size measurements indicated a mean
particle size of 1060 nm with a standard deviation of
106%. This emulsion degraded in a matter of days.

EXAMPLE 6
        The particle size distribution of an emulsion
10   can be measured by centrifugation. A sample of the
emulsion of Example 19 below was placed in the Horiba
CAPA-700 Particle Analyzer (Horiba Instruments,
Irvine, CA). The particle size distribution, based
on assuming the particles have a density of 1.66 g/cu
15   cm, was as follows:

Particle Size Range microns	Volume Percent
0.0-0.5	12
0.5-1.0	26
1.0-1.5	22
1.5-2.0	15
2.0-2.5	7
2.5-3.0	0

EXAMPLE 7
25   The long term stability of the emulsions of the
present invention was determined. The emulsion
described in Example 19 below was placed at 19°C and
the particle size determined at intervals using the
Nicomp 370. The results are contained in the
30   following table:

Time (days)	Mean Particle Diameter nm
5	194
13	216
19	245
27	258
33	289
41	283

47	306
61	335
89	305

5   This emulsion initially grew rapidly from 194 to 289 nm over the first month. However, since then the growth has largely stopped. Extrapolation of the curve of a graph of diameter vs time supports at least a one year stability for this emulsion.

EXAMPLE 8

10   The emulsion of Example 42 below was used to test the imaging capabilities of these colloidal dispersions administered by various routes. An approximately 20 kg mongrel dog was anesthetized with sodium barbiturate, and prepared for ultrasound

15   examination according to the method described in Example 38.

A 0.2 mL/kg intravenous injection produced a strong contrast signal in the right and left ventricles of the heart within the first minute

20   following the injection. Doses of 0.5 mL/kg produced a strong Doppler signal in all organs examined, including the vascular system, liver, kidneys, heart, and vessels of the central nervous system.

A 0.5 mL injection either by an intradermal,

25   intracutaneous, or intramuscular route caused local contrast, permitting examination of the musculoskeletal system.

A 1000 mL solution, prepared by diluting 50 mL of the emulsion of Example 42 into 950 mL of saline,

30   was given by the oral route, effectively providing an intragastric and intraduodenal intraluminal administration. The lumen of the gastrointestional system was enhanced, providing better visualization of the liver, spleen, and internal reproductive

35   organs.

A 10 mL volume of the emulsion of Example 42
below was administered by the intracystic route,
affording enhanced visualization of the urinary
bladder.

5   The above specific examples could be used to
provide useful ultrasound contrast with the colloidal
dispersions of the present invention by additional
routes of administration. Specifically, the
emulsions could be given by any of the following

10   routes, among others: intraabdominal, intraarterial,
intraarticular, intracapsular, intracervical,
intracranial, intraductal, intradural, intralesional,
intralocular, intralumbar, intramural, intraocular,
intraoperative, intraparietal, intraperitoneal,

15   intrapleural, intrapulmonary, intraspinal,
intrathoracic, intratracheal, intratympanic,
intrauterine, and intraventricular. Methods for
administration by these routes can be found in a
standard radiology text, such as "Pharmaceuticals in

20   Medical Imaging," edited by DP Swanson, HM Chilton,
JH Thrall. MacMillian Publishing Co., Inc., 1990,
which text is incorporated herein by reference.

        In addition to the above indicated organs or
organ systems studied, one could study the lungs,

25   breast, prostate, and endocrine systems by known
means. The kinds of medical conditions amenable to
study with the agents of the present invention are
numerous. They include metabolic, traumatic,
congenital, neoplastic, or infectious diseases. A

30   description of the use of ultrasound imaging in these
conditions can be found in the text "Diagnostic
Ultrasound," edited by CM Rumack, SR Wilson, JW
Charboneau, Mosby Year Book, Boston, 1991,
incorporated herein by reference.

35   EXAMPLE 9

The colloidal dispersions of the present invention can produce a contrast effect in the ultrasound signal at concentrations ranging from 0.00001% w/v to 166% w/v.

5   If a 1% emulsion (such as the emulsion of Example 42) is diluted ten-fold (by adding one mL to nine mL of buffer) and a 0.1 mL aliquot added to 1000 mL water at 37°C and the ultrasound intensity measured, there is a substantial increase in the

10   backscatter. Specifically, the signal intensity, measured with the system described in Example 1, increases from 2.7 to 9.8 within the first minute following the above addition. At a greater dilution, the backscatter is indistinguishable from background.

15   Thus, the lower limit for the concentration of the dispersed phase material is 0.00001%.

If 5 mL of dodecafluoropentane is added to 5 mL of water containing the surfactant mixture described in Example 25 below, and the suspension comminuted

20   for 5 minutes by the method of Example 4, a 166% w/v emulsion is formed. This can be immediately administered, for example orally, to an organism to afford excellent ultrasound contrast. This amount represents a high end to the concentration of the

25   dispersed phase material because higher concentrations produce formulations which tend to be unstable.

EXAMPLE 10

Proteins can be used to stabilize the colloidal

30   dispersions of the present invention. Using high-intensity ultrasound, one can synthesize aqueous suspensions of proteinaceous microspheres filled with nonaqueous liquids (i.e., microcapsules). These are distinct from the ultrasound contrast agents of U.S.

35   Pat. Nos. 4,718,433 and 4,774,958, which contain only

gases, and follow the methods described by Suslick
and Grinstaff (Suslick KS, Grinstaff MW:Protein
microencapsulation of nonaqueous liquids. _J Amer
Chem Soc 112_:7807-7809, 1990). This reference

5   describes only the use of high boiling nonaqueous
liquids (which are unsuitable as ultrasound contrast
agents) and fails to disclose the use of either low
boiling liquids in general, or organic halides, in
particular, as the nonaqueous liquids.

10   Proteinaceous microspheres can be synthesized
with a high intensity ultrasound probe (Heat Systems,
W375, 20 kHz, 0.5 in. Ti horn) from human serum
albumin or hemoglobin. Typically, 5% pentane or 3%
diethyl ether and 5% albumin are irradiated for three

15   minutes at an acoustic power of about 150 W/sq cm, at
23°C and a pH of 7.0. The resulting dispersion has a
Gaussian distribution and a mean particle diameter of
about 2.3 microns. They maintain their particle size
for up to two months at 4°C.

20   In addition to albumin or hemoglobin, the
following proteins can be used: alpha-1-antitrypsin,
alpha fetoprotein, aminotransferases, amylase, C-
reactive protein, carcinoembryonic antigen,
ceruloplasmin, complement, creatine phosphokinase,

25   ferritin, fibrinogen, fibrin, transpeptidase,
gastrin, serum globulins, myoglobin, immunoglobulins,
lactate dehydrogenase, lipase, lipoproteins, acid
phosphatase, alkaline phosphatase, alpha-1-serum
protein fraction, alpha-2-serum protein fraction,

30   beta protein fraction, gamma protein fraction, gamma-
glutamyl transferase.

          In addition to pentane or diethyl ether, other
aliphatic hydrocarbons, organic halides, and ethers
can be used as described above for pentane.

35   EXAMPLE 11

The relationship of the size of the particles of the colloidal dispersion as an emulsion or microemulsion and the size of the microbubbles formed upon phase shift can be determined.

5   An aliquot of the emulsion of Example 27 below was placed in the Nicomp 370, operating at 19°C and the mean particle size of the liquid emulsion was determined to be 231.7 nm. The temperature control of the instrument was adjusted to 37°C and after

10   temperature equilibration, which took about five minutes, the particle size was redetermined. The microbubble dispersion formed had a mean particle size of 1701.5 nm, an increase in size of 7.34-fold.

One can also calculate the expected change in

15   dispersion size if one knows the relative densities of the dispersed liquid as a gas and liquid. For example, the Gas Data Book, by W Braker and A Mossman, Matheson, contains such data. Examining octafluorocyclobutane, one finds that 1 L of the

20   liquid yields 188 L of gas at a pressure of 760 mm Hg and 15°C. Since the volume of a sphere is related to the diameter of a sphere by the cubic root of the volume, the phase transition for an octafluorobutane emulsion particle will cause a 5.7-fold increase in

25   diameter.

EXAMPLE 12

The safety of the emulsions of the present invention is dramatically demonstrated in the mini-pig. Albunex brand ultrasound contrast agent, under

30   development and the subject of U.S. Pat. Nos. 4,718,433 and 4,774,958, shows grave hemodynamic effects in the pig (Ostensen J, Hede R, Myreng Y, Ege T, Holtz E.) Intravenous injection of Albunex microspheres causes thromboxane mediated pulmonary

35   hypertension in pigs, but not in monkeys or rabbits.

23

Acta Physiol Scand 144:307-315, 1992). At doses as
low as 0.001-0.05 mL per kg hypotension results. One
pig died after a slow infusion of 0.05 mL per kg.

5   An experiment was performed in a 30 kg mini-pig
under halothane anesthesia, using the protocol of the
above reference. The results are contained in the
following table:

Dose, mL/kg	Cumulative Dose, mL/kg	Hemodynamic Effect
0.01	0.01	None
0.02	0.03	None
0.05	0.08	None
0.10	0.18	None
0.20	0.38	None
0.30	0.68	None
0.40	1.08	None
0.50	1.58	None
0.60	2.18	None
0.60	2.78	None
0.80	3.58	None
0.30	3.88	None
2.00	5.88	labored breathing

All doses provided good cardiac contrast. The
doses above 0.4 mL/kg provided Doppler enhancement of
25   the liver as well.

In conclusion, injections of an emulsion of the
present invention at 40-times the lethal dose of
albumin microspheres in the mini-pig had minimal,
transient effects. The threshold dose for an effect
30   with Albunex is 0.001 mL per kg of the albumin
microspheres or 2000-times below the threshold dose
for an effect of the colloidal dispersions of the
present invention.

EXAMPLE 13
35   The selection of amphiphilic materials with the
proper hydrophilic-lipophilic balance (HLB) number
for the selected dispersed phase is important for the
stability of the colloidal dispersion. One way to

determine the HLB number is to measure the
interfacial tension of various surfactant mixtures.
(A good general review of the HLB method can be found
in: Emulsions: Theory and Practise, Paul Becher,
5 referred to above, pp. 232-252.

Mixtures of Pluronic P-123 and Pluronic F-127
were formed, yielding a 1% solution, v/v, with graded
HLB numbers and the interfacial tension (IFT) of the
solutions against dodecafluoropentane determined at
10 4°C, using a Kruss Drop Volume Tensiometer DVT-10,
Kruss USA, Charlotte, NC. The results are contained
in the following table:

RELATIONSHIP BETWEEN HLB AND INTERFACIAL TENSION

P-123	F-127	HLB	IFT (dynes/cm)
1.00	0.00	8	27.07
0.86	0.14	10	23.94
0.75	0.25	12	23.58
0.60	0.40	14	22.48
0.50	0.50	15	22.80
0.40	0.60	16	23.16
0.25	0.75	19	23.61
0.00	1.00	22	26.36

The above data, when graphed, indicate an HLB
for dodecafluoropentane of about 14. The use of
25 amphiphilic materials, such as anionic, nonionic,
cationic, or zwitterionic surfactants with an HLB
number of 14 will provide the greatest stability for
emulsions of the above liquid dispersed phase.

EXAMPLE 14
30 The interfacial tension between the liquid
dispersed phase and the liquid continuous phase can
be used to develop formulations, since this property
has a significant influence on the stability of the
colloidal dispersion.

The Ostwald ripening theory predicts a strong
dependence of particle size stability on interfacial
tension (reviewed by Kabalnov AS, Shchukin ED;
Ostwald ripening theory: Applications to
fluorocarbon emulsion stability, Advances in Colloid
and Interface Science, 38:69-97, 1992, incorporated
herein by reference). The theory predicts stability
and interfacial tension are inversely proportionate
to each other. For example, if one can add
amphiphilic materials which provide a five-fold
lowering of interfacial tension, one will obtain a
five-fold increase in stability.

Interfacial tensions of various amphiphilic
materials in aqueous solutions (all expressed as v/v
solutions) against dodecafluoropentane were measured
at 4°C and emulsions created from each formulation,
as described in Example 13.

Pluronic P-123, 1%, and dodecafluoropentane had
an interfacial tension of 27.1 dynes/cm and did not
form a stable emulsion.

Pluronic F-127, 1%, and dodecafluoropentane had
an interfacial tension of 26.4 dynes/cm and did not
form a stable emulsion.

Zonyl FSO, 1%, and dodecafluoropentane had an
interfacial tension of 5.8 dynes/cm and formed a
stable emulsion.

Pluronic P-123, 0.33%, Pluronic F-127, 0.33%,
and Zonyl FSN, 0.33%, and dodecafluoropentane had an
interfacial tension of 14.1 dynes/cm and did form a
stable emulsion.

Pluronic P-123, 1%, Zonyl FSO, 1.0%, sodium
chloride, 1%, and sodium perfluorooctanoate, 0.5%,
and dodecafluoropentane had an interfacial tension of
2.71 dynes/cm and formed a stable emulsion.

Thus, amphiphilic materials with interfacial
tensions below 26 dynes/cm were required to form

stable emulsions. Related findings would be obtained
with other organic halides or with aliphatic
hydrocarbons or ethers.

EXAMPLE 15

5.   The viscosity of the liquid continuous phase can
be used to develop formulations, since this property
has a significant influence on the stability of the
colloidal dispersion.
          The Ostwald ripening theory predicts a strong

10   dependence on particle size stability and viscosity
(see Kabalnov AS, et al. in Example 14). The theory
predicts stability and viscosity are directly
proportionate to each other. For example, if one can
add viscogens (viscosity enhancing agents) which

15   provide a five-fold increase in viscosity, one will,
in general, obtain a five-fold increase in stability.
          Examples of viscogens include, but are not
limited to, carboxymethylcellulose, sorbitol,
iohexol, other iodinated x-ray contrast materials,

20.   dextrose, polyethylene glycols. The emulsion of
Example 38 below was prepared with or without 5%
polyethylene glycol (PEG) 200, which produced a
viscosity of 1.1 cP, and stability noted. The
emulsion containing 5% PEG 200 had greater stability.

25   EXAMPLE 16
          The ultrasound backscatter from dispersions of
the emulsions of Examples 44 and 18 below were
measured with a Hewlett Packard Model 77020
ultrasound scanner to determine the relative potency

30   of the phase shift colloids of the present invention,
which are liquid-liquid emulsion dispersions at room
temperature but which become microbubbles following
administration with either stable emulsions, as
described by Long and others (U.S. Patents 7,767,610,

4,987,154, and JP 2196730), Davis and others (EP
245019), and JP Patent 1609986 and JP 63060943), or
with true air microbubbles, as described in EP
467031, EP 458745, WO 9115244, US 5088499, 5123414,
5   US 4844882, US 4832941, US 4466442, and US 4276885,
each of which is incorporated herein by reference.

        The air microbubbles were created by the
following procedure. Introduce 0.5 mL of air into a
10 mL syringe and 10 mL of a 1.0%, v/v, solution of
10   Pluronic F-68 into another 10 mL syringe, which is
connected to the first syringe by a three-way
stopcock. Pass the liquid and air back and forth
between the two syringes rapidly. After about five
passes the air and liquid have mixed and the solution
15   has a milky, white appearance. Continue mixing for a
total of 20 passes. A 1.0 mL sample of the gas
dispersion added to 250 mL of water gave an
ultrasound image with an intensity similar to hepatic
tissue (4+ strength). Surprisingly, the intensity of
20   the ultrasound backscatter produced by the air
microbubbles decreased rapidly, so that within five
minutes the backscatter had returned to base line.
This lack of persistence limits the diagnostic
utility of air microbubbles.

25   On the other hand, 1.0 to 10.0 mL of a
perfluorohexane emulsion in 250 mL of water at 37°C
yielded an ultrasound image similar to flowing blood
(0-1+ strength), indicating that these formulations
produce ultrasound contrast only at extremely high
30   dosages, which limit their general utility.

        A 1.0 mL sample of the dodecafluoropentane
emulsion diluted in 250 mL of 37°C water yielded an
ultrasound image with the intensity of the
microbubble solutions (4+ strength) which persisted
35   for over 10 minutes, a time sufficient to be
diagnostically useful.

Parenthetically, all three experimental
solutions were visually cloudy solutions of nearly
equal apparent turbidity. These experiments
demonstrate that the ultrasound contrast agents of
5   the present invention show greater persistence and/or
potency than the prior art ultrasound contrast agents
to a diagnostically useful extent.

EXAMPLE 17

A 1.0 mL sample of the contrast agent of Example
10   19 was withdrawn from a vial with a 1.0 mL syringe
equipped with a 21-gauge needle and approximately 0.2
mL placed on a glass slide. A glass cover slip was
placed over the liquid and the sample placed on the
stage of a light microscope equipped with an eye
15   piece micrometer, a temperature-controlled chamber, a
35-mm camera, and a Panasonic video camera.

The emulsion was examined under oil-immersion at
20°C. At this temperature the emulsion consisted of
0.2-0.3 micron particles which were undergoing rapid
20   Brownian motion.

The temperature control was changed to 37°C and
the emulsion observed and images recorded. As the
temperature rose the particles would individually
suddenly grow in size until at 37°C the emulsion had
25   become a collection of 1-3 micron bubbles. The
bubbles, in distinction to the liquid emulsion, were
easily deformable. They did not, however, appear to
coalesce. After 40 minutes of experimentation the
microbubble ensemble remained intact and stable.

30   EXAMPLE 18

The criticality that some portion of the liquid
dispersed phase undergo a liquid to gas phase
transition at the body temperature of the organism to
be imaged, in this case using an example temperature

29

of 37°C, to the utility as an ultrasound contrast
agent was tested by subjecting a series of emulsions,
each with different liquid dispersed phases, to
ultrasound imaging at 37°C.

5   The following emulsions were formulated or
obtained from sources and 1.0 mL aliquots placed in
1000 mL of water at 37°C. The emulsion formed with
1-iodoperfluorooctane was formulated according to the
methods disclosed by Long and others (U.S. Patents

10   4,767,610, 4,987,154 and JP 2196730). The emulsion
with perfluorodecalin was formulated according to the
disclosures of JP Patent 1609986 and JP 63060943.
The emulsion with triolean was formulated according
to methods disclosed by Davis and others (EP 245019).

15   The contents of each of these patents are hereby
incorporated by reference. Ultrasound images were
obtained of the solution before and after the
addition and the results expressed as a percentage of
enhancement times the length of time over which

20   enhancement was observed.

Dispersed Phase	Amphiphilic Material/Class D.P.	Boiling Point (°C)	Enhancement Percent-Minutes X1000
Decafluorobutane	Octadecylamine HCl/Cationic	-5.8	625
Dodecafluoropentane	Poloxamer-Zonyl/Nonionic	29	740
Perfluorohexane	Dodecylsulfate/Anionic	59	178
Perfluorooctane	Poloxamer-Zonyl/Nonionic	98	24
Perfluorodecalin	Poloxamer-Phospholipid-Oleate /Mixed	141	8
1-iodoperfluorooctane	Phospholipid/Zwitterionic	160	6
Triolean	Phospholipid/Zwitterionic	235	0.2
Saline	Not Applicable	Shaken	0.006

As indicated above, the preferred formulations
are the emulsions which undergo a complete phase

35   shift at or below 37°C. The high vapor pressure
liquids perfluorohexane and perfluorooctane which
have vapor pressures at ambient temperature above 20
Torr, provided some contrast when compared to
agitated saline or perfluorodecalin which has a vapor

pressure at ambient temperature below 20 Torr. This
may indicate some advantage with respect to the use
of these compounds as ultrasound contrast agents,
however the mechanism for enhancement by these
5   materials is not fully understood and is not
considered practically useful relative to those
materials which boil at about 40°C or below.

EXAMPLE 19
        The ultrasound contrast agents of the present
10   invention can be made with the following equipment
        and steps: Microfluidizer, Model 110Y, Interaction
chamber pressure 14,000 PSI; Pressure vessels, 316
steel, 5 L and 12 L sizes; Filters, cellulose
acetate, 0.22 micron; Filter holders, 142 mm. The
15   following solutions were made: 25% (w/v) sorbitol,
12 L; 2.5% w/v sodium perfluorooctanoate (PCR, Inc.,
Gainsville, FL); 60 g Pluronic P-123, 60 g Zonyl FSO,
7 mL 2.5% sodium perfluoro-octanoate solution, 1 L;
sonicate to aid dissolution (stock surfactant
20   solution). The Microfluidizer was primed with the
sorbitol solution. The interaction chamber, tubing,
and cooling coil are covered with chipped ice during
the comminution process. To a 5 L pressure vessel
with stir bar in an ice bath add sequentially: 500
25   mL sorbitol solution; 500 mL stock surfactant
solution; 800 mL water; 200 g dodecafluoropentane.
Pressurize vessel to 10 PSI with nitrogen for 45 min.
Pass the suspension through the Microfluidizer for 45
min at 14,000 PSI. Transfer the emulsion to a vessel
30   containing 8 L of 25% sorbitol at 4°C and mix well.
Transfer the emulsion to 100 mL vials using positive
pressure, passing the material through a 0.22 micron
filter in the process. Cap and seal the vials. The
amphiphilic materials of this Example, including
35   fluorine-containing surfactants and polyoxypropylene-

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polyoxyethylene glycol nonionic block co-polymers,
produce a formulation with acceptable stability.

EXAMPLE 20

A 0.4 ml portion of n-pentane (Aldrich Chemical,
5   Milwaukee, WI) was added to 2.0 mL of water at 4°C.
Two clear separated phases resulted. NaCl was added
(0.4 mL of a 10% w/v solution) to make a total of
2.8 mL. Approximately 135 mg of phosphatidyl
lecithin (Sigma Chemical, St. Louis, MO) was added
10·   with stirring and the resulting slurry mixed by
vigorous vortex agitation. The milky white solution
separated into two phases within 5 min. upon
standing. Ethanol was added in 0.1 mL increments
with mixing to a total of 1.74 mL. There was no
15   change in the appearance of the two-phase mixture.
The formulation of this Example showed good in vitro
ultrasound backscatter characteristics and
demonstrates the use of aliphatic hydrocarbons having
six or fewer carbon atoms and 17 total atoms.

20   EXAMPLE 21

A milky suspension was formed by adding together
1.80 mL water, 0.2 mL 10% NaCl, 0.1 mL ethanol, and
100 mg lecithin. A 0.1 mL portion of
dodecafluoropentane (PCR, Gainsville, FL) was added
25   and following mixing two phases were obtained. A 0.1
mL portion of n-pentane was added and then 0.2 mL
dodecafluoropentane aliquots were added to bring the
total dodecafluoropentane to 20% v/v. The resulting
suspension was mixed and three phases obtained, two
30   milky phases and a small clear phase. Additional
NaCl was added to bring the solution to 7% and a 1 mL
aliquot of ethanol added with no change in the
character of suspension. The formulation of this
Example showed good in vitro ultrasound backscatter

characteristics and demonstrates the use of mixtures of a hydrocarbon and a fluorocarbon.

EXAMPLE 22

To a 2.0 ml portion of dodecafluoropentane was added 330 mg of lecithin. Following mixing, 1.0 mL of water was added and the suspension further mixed. A milky colloidal dispersion was formed. A milky colloidal dispersion was formed, demonstrating the use of a single surfactant as the amphiphilic material, in this case a naturally-occurring zwitterionic surfactant. Useful contrast agents would also be formed by replacing the portion of dodecafluoropentane in the formulation with an ether, and diethyl ether was specifically found to provide a useful contrast signal. Related compounds, such as methyl ether, and vinyl ether are expected to be useful as well.

EXAMPLE 23

A 0.46 g portion of sodium dodecylsulfate (SDS) was added to 0.72 mL water and 8.00 mL dodecane. A 1.47 mL aliquot of pentanol was slowly added. Initially the suspension contained white, "filamentous" SDS in a clear fluid. A 1.0 mL addition of pentanol and gentle mixing lead to a substantial dissolution of the SDS. A 0.5 mL addition of pentanol with mixing lead over 10-15 min at room temperature to a clear, monophasic microemulsion. This formulation produced rather poor acoustic backscatter, demonstrating that a colloidal dispersion containing a liquid dispersed phase with a boiling point greater than about 40°C, here exemplified by dodecane (b.p.216°C), is unsuitable as an ultrasound contrast agent within the meaning of the present invention.

EXAMPLE 24

The composition of the water, pentanol, dodecane, sodium dodecylsulfate microemulsion of Example 23 was varied to determine the compositional boundaries of the microemulsion. The following mixtures were prepared at room temperature and the appearance following 30 min. of stirring was noted:

Volume of Addition (mL)

EXPERIMENT	WATER	PENTANOL	DODECANE	SDS	APPEARANCE
5-1	1.00	1.00	1.00	372 mg	Clear
5-2	1.10	1.00	1.00	372 mg	Clear
5-3	1.20	1.00	1.00	372 mg	Clear
5-4	1.30	1.00	1.00	372 mg	Clear
5-5	1.50	1.00	1.00	372 mg	Milky
5-6	1.50	1.10	1.00	372 mg	Milky
5-7	1.50	1.30	1.00	372 mg	Milky
5-8	1.50	1.50	1.00	372 mg	Slt. Milky
5-9	1.50	1.60	1.00	372 mg	Clear, Bluish Cast

The 5-9 microemulsion became milky upon heating (greater than about 45°C) and became clear, with a bluish cast, again upon cooling to room temperature. This reversible change in appearance could be repeated through at least six temperature shift cycles.

EXAMPLE 25

A 0.51 mL portion of octyl amine (Sigma Chemical Corp., St. Louis, MO) was added to 1.0 mL of water to form a clear solution. A 1.0 mL portion of octane was added and the clear solution became milky. A 0.49 mL portion of octanoic acid was added and the solution became a gel. A 0.17 mL aliquot of a 3.6 M KOH solution dissolved the gel to produce a clear microemulsion. Five additions of water in 0.1 mL aliquots with mixing continued to yield a clear

microemulsion. The sixth addition converted the
clear emulsion to a milky colloidal dispersion. This
Example demonstrates the formulation of an aliphatic
hydrocarbon-containing emulsion with amphiphilic
5   material comprising cationic surfactants.

EXAMPLE 26
        A 1.0 mL portion of dodecafluoroheptanol (PCR)
was added to 1.0 mL of dodecafluoropentane to form a
clear, homogenous solution. The same quantity of
10   octafluoropentanol in dodecafluoropentane yielded two
clear, non-mixing phases. The addition of 2.0 to 4.0
mL water to the dodecafluoroheptanol-
dodecafluoropentane yielded two non-mixing phases.
Upon cooling to 4°C the two clear phases changed to
15   three clear phases.

EXAMPLE 27
        A solution of 10% (v/v) Fluorad FC-430 (3M
Chemical, St. Paul, MN) in water was prepared by
adding 10 mL FC-430 to 100 mL water at room
20   temperature and mixing. To 5 mL of this solution 1.0
mL dodecafluoropentane and 1.0 mL octafluoropentanol
was added to yield an emulsion.

EXAMPLE 28
        A 2.0 ml portion of 10% v/v FC-430 solution was
25   added to 2.0 mL dodecafluropentane and two phases
resulted. The addition of 0.3 mL
dodecafluoroheptanol yielded a milky, white emulsion.

EXAMPLE 29
        A 1 mL portion of 1.26 M 2-amino-2-methyl-1-
30   propanol (AMP) perfluorooctanoate was added to 1.0 mL
of dodecafluoropentane, and 1 mL of 25% Pluronic F68
to yield two phases of milky liquid. A 0.05 mL

addition of dodecafluoroheptanol yielded a single
phase colloidal dispersion.

EXAMPLE 30

5   A 2.0 mL portion of a 15% (v/v) Pluronic F68
solution was added sequentially to 2.0 mL
dodecafluoropentane and 0.2 mL dodecafluoroheptanol
on ice. The mixture was taken up in a 5 mL glass
syringe connected to a three-way stopcock and a
second 5 mL glass syringe and forcefully passed back
10   and forth between the syringes to yield a thick white
emulsion.

EXAMPLE 31

The following mixture was formed by sequential
addition at 4°C: 2.0 mL 15% Pluronic F68, 2.0 mL
15   dodecafluoropentane, 2.0 mL 0.2M AMP
perfluoroctanoate, 0.1 mL dodecafluoroheptanol. The
mixture was taken up in a 5 mL glass syringe
connected to a three-way stopcock and a second 5 mL
glass syringe and forcefully passed back and forth
20   between the syringes to yield a thick white emulsion.

EXAMPLE 32

The following mixture was formed by sequential
addition at 4°C: 2.0 ml 15% Pluronic F68, 0.42 g D-
sorbitol (Sigma) dissolved in 0.5 mL H₂O, 0.2 mL
25   dodecafluoroheptanol, and 2.0 mL dodecafluoropentane.
The mixture was taken up in a 5 mL glass syringe
connected to a three-way stopcock and a second 5 mL
glass syringe and forcefully passed back and forth
between the syringes to yield a thick white emulsion.

30   EXAMPLE 33

The following mixture was formed by sequential
addition at 4°C: 2.0 mL of 15% (v/v) Pluronic F-68,

0.40 mL 0.1 M Tris(hydroxymethyl) amino methane
(Tris) perfluorooctanoate, pH 7.2, 2.0 mL
dodecafluoropentane. The mixture was taken up in a 5
mL glass syringe connected to a three-way stopcock
5   and a second 5 mL glass syringe and forcefully passed
back and forth between the syringes to yield a white
colloidal dispersion.

EXAMPLE 34
          The following mixture was formed by sequential
10   addition at 4°C: 60 mL 25% Pluronic F68, 24 mL 1,1,7-
H-dodecafluoroheptanol, 75.8 g dodecafluoropentane.
The mixture was comminuted by batchwise mixing using
30 cc syringes, a three-way stopcock and 40 manual
passages. The mixture was sequentially diluted 1:10
15   twice with a solution composed of 8.0 mL 25% Pluronic
F68, 2.0 mL 50% D-sorbitol, 1.0 mL pH 7.2, 0.1 M Tris
perfluorooctanoate and further comminuted by syringe
passage. This formulation was administered to mice,
weighing 20-30 g, intravenously by tail vein
20   injection and observed for seven days. The results
are contained in the following table:

DOSAGE (mL/kg)	OBSERVATIONS
20	Survival
25	Morbid but survival
30	Morbid but survival
40	No Survival

          This biocompatible colloidal dispersion was
stable for at least two weeks after formulation.

EXAMPLE 35
30   The following formulation was prepared: 1.0 mL
25% polyethylene glycol 3550, 1.0 mL 50% sorbitol,
3.0 mL 15% (w/v) Pluronic F-68, 3.0 mL 20% (w/v)

Fluorosurfactant FC 430, 0.4 mL 0.1 M Tris
perfluorooctanoate and 1.0% (v/v)
dodecafluoropentane. The mixture was comminuted in a
water bath sonicator by the application of acoustic

5   energy at 4°C for 10 min to yield a milky colloidal
dispersion.

EXAMPLE 36

    A series of solutions of aqueous media, each
containing different proportions of amphiphilic

10   materials, were formed and tested as the basis for a
formulation.

    Solution A: A clear solution containing 6.0 mL
of a 25% solution of Pluronic F-68, 6.0 mL of a 50%
solution of PEG3350, 0.60 mL 0.1 M Tris

15   perfluorooctanoate, and 2.4 mL H_2O.

    Solution B: A clear solution containing 1.18 mL
of a 25% solution of Pluronic F68, 6.0 mL of a 50%
solution of PEG 3350, 0.12 mL Tris perfluorooctanoate
and 7.7 mL H_2O.

20   Solution C: A turbid solution, containing a
gelled precipitate, was obtained by mixing 6.0 mL of
50% PEG 3350, 0.75 mL Tris perfluorooctanoate and 1.5
mL H_2O. This solution is not biocompatible for
intravascular administration but is biocompatible for

25   oral, intraperitoneal, rectal or intrauterine
administration.

    Solution D: A clear solution was obtained by
mixing 6.0 mL 25% (w/v) Pluronic F-68, 6.0 mL 50%
(w/v) PEG 3350, 0.6 mL 0.1M Tris perfluorooctanoate

30   and 2.4 mL H_2O.

    Solution E: A clear solution was obtained by
mixing 6.0 mL 50% (w/v) PEG 3350, 7.5 mL 20% (w/v)
FC-430, 0.75 mL Tris perfluoroctanoate and 0.75 mL
H_2O.

Solution F: A clear solution was obtained by
mixing 1.8 mL 25% (w/v) Pluronic F-68, 6.0 mL 50%
(w/v) PEG 3350, 0.12 mL 0.1M Tris perfluorooctanoate,
and 7.7 mL H₂O.

5   Solution G: A clear solution, containing a tiny
precipitate was formed by mixing a 3.0 mL Pluronic
F-68 3.75 mL (w/v) FC-430, 6.0 mL PEG 3350, 0.68 mL
Tris perfluorooctanoate, and 1.57 mL H₂O.

          To 7.0 mL of solutions A-G a 0.14 mL portion of
10   dodecafluoropentane was added at 4°C. The colloidal
dispersions were created by 40 passes between two
syringes using a three-way stopcock.

          Formulation D was administered to mice via tail
vein injection and had a LD50 of 20 ml/kg.
15   Formulations F and G were toxic at 10 ml/kg.

EXAMPLE 37

          An emulsion was formulated by mixing 45 mL of
20% PEG 3350, 237 mg Pluronic F68, 0.225 mL Fluorad
FC-171, 2.25 mL 0.1 M Tris perfluorooctanoate, and
20   10% (v/v) dodecafluoropentane. This was comminuted
by mixing in a two-syringe, three-way stopcock
apparatus.

          This formulation was biocompatible in a test of
hemolysis. Whole blood was collected from a rat by
25   intracardiac puncture (2.0 mL) in a EDTA-containing
evacuated collection tube. A 0.10 mL aliquot of
blood was added to a 0.20 mL aliquot of the above
formulation to simulate the peak blood level obtained
following an intravenous dosage of 100 mL/kg. The
30   blood was mixed with the formulation for two minutes
and the sample centrifuged. The supernatant was
clear, the pellet deep red, indicating no hemolysis
even at this extremely large dosage.

          This formulation was also biocompatible in a
35   test of acute toxicity by causing only minor, labored

breathing in mice after intravenuous administration
at 20 mL/kg.

EXAMPLE 38

A formulation containing dodecafluoropentane and
5 amphiphilic materials in an aqueous media was tested
for biocompatibility and utility as an ultrasound
contrast agent. A stock solution of 90 mL of 20% PEG
3350, 474 mg of Pluronic F-68, 0.45 mL Flurorad
FC-171, and 4.5 mL 0.1 M Tris perfluorooctanoate was
10 mixed and yielded a clear solution. To 9.0 mL of
above was added 0.18 mL of dodecafluoropentane. A
colloidal dispersion was formed by comminution
between two 5 mL syringes.

An echocardiology study was performed in a 32 kg
15 dog according to the model described by Keller MW,
Feinstein SB, Watson DD: Successful left ventricular
opacification following peripheral venous injection
of sonicated contrast: An experimental evaluation.
Am Heart J 114: 570d (1987), incorporated herein by
20 reference. Eleven administrations of the above
formulation were given intravenously at doses of 0.05
to 0.75 mL/kg. The 0.05 mL/kg dose gave only slight
contrast enhancement of the right and left ventricles
immediately following injection. All doses between
25 0.10 and 0.75 mL/kg gave diagnostically useful
enhancement of the ventricular chambers. The
injections had a minimal effect on hemodynamic
parameters.

A 10% dodecafluoropentane emulsion was formed in
30 the above formulated aqueous media and the contrast
enhancement produced compared to the 2% formulation.
At doses of 0.20 and 0.25 mL/kg this formulation
produced intense cardiac chamber opacification
following intravenous administration with minimal
35 hemodynamic changes.

40

EXAMPLE 39

An emulsion containing a high density, high
viscosity biocompatible aqueous medium as the
continuous phase was formulated. It contained 0.06
5   mL of 15% Pluronic F68, 0.06 mL Zonyl FSO-100, 0.12
mL of 5% Zonyl FSN-100, 0.146 mL of 0.1M Tris
perflurooctanoate, pH 7.2, 4.47 mL of 76% w/v iohexol
(Omnipaque 350, Sterling Winthrop, New York), and 0.6
mL of dodecafluoropentane. A stable formulation was
10   formed following comminution by 2-syringe mixing.
Other high density iodinated x-ray contrast materials
could be substituted for iohexol such as iopamidol,
ioversol, iopentol, iodiximol, and other related
compounds. Use of water alone as the continuous
15   phase medium yielded contrast agents which settled
rapidly following formulation in the bottle. This
example demonstrates the utility of a high density,
high viscosity biocompatible aqueous medium as the
continuous phase.

20   EXAMPLE 40

A series of polyoxypropylene-polyoxyethylene
glycol nonionic block copolymers were tested for
their ability to act as amphiphilic materials in
stabilizing the formulations of dodecafluoropentane
25   liquid-liquid emulsions. The following solutions
were formed:

A- 1.9 mL of 25% Pluronic F-68 and 0.04mL
dodecafluoropentane

B- 1.9 mL of Pluronic L-121 and 0.04ml
30   dodecafluoropentane

C- 1.9 mL of Pluronic L-122 and 0.04mL
dodecafluoropentane

D- 1.9 mL of Pluronic L-121 and 0.04mL
dodecafluoropentane

E- 1.9 mL of Pluronic L-101 and 0.04mL
dodecafluoropentane

F- 1.9 mL of Pluronic L-92 and 0.04mL
dodecafluoropentane

5   G- 1.9 mL of Pluronic L-81 and 0.04mL
dodecafluoropentane

H- 1.9 mL of Pluronic P-123 and 0.04mL
dodecafluoropentane

The above solutions were placed in sealed glass
10 tubes and vortex mixed at 4°C for 10 min. The size
and number of the dispersed dodecafluoropentane phase
particles was accessed visually. Solution H yielded
the smallest particles.

EXAMPLE 41

15   The relative hydrophilic-lipophilic balance
(HLB) is a method of optimizing a nonionic surfactant
solution to achieve greatest stability. It is
described in detail in Emulsions: Theory and
Practice, Paul Becher, 1965, Robert E. Krieger
20 Publishing Company Malabar, FL, and references
contained therein, and is incorporated here by
reference. Solutions of Pluronic L61 (HLB 3.0) and
F68 (HLB 29) were mixed to achieve intermediate HLB
values by the following formula:

25   $HLB = f_{L61}$ {HLB of L61} + f_{F68} {HLB of F68}
The actual solutions, the calculated HLB values, and
the stability of the final formulation (a 2% v/v
emulsion of dodecafluorohexane) are contained in the
following table:

PLURONIC L61	PLURONIC F68	RELATIVE HLB	STABILITY
9.6mL	0.4mL	4	0
8.8	1.2	6	+++
8.1	1.9	8	+++
7.3	2.7	10	+
6/5	3.5	12	0
5.8	4.2	14	0
5.0	5.0	16	0
4.2	5.8	18	0

10   O = no stability; + = some stability; +++ = greatest
        stability

        The relative HLB for perfluorohexane established
        by this work is 6-8. The greatest stability of
        perfluorohexane emulsions will be achieved by using
15   amphiliphic materials with relative HLB values of
        6-8, regardless of their chemical structure.

        EXAMPLE 42
        A large scale formulation of ultrasound contrast
        agents of the present invention can involve the
20   following equipment and steps: Microfluidizer, Model
        110Y, Interaction chamber pressure 14,000 PSI;
        Pressure vessels, 316 steel, 5 L and 12 L sizes;
        Filters, cellulose acetate, 0.22 micron; Filter
        holders, 142 mm. The following solutions were made:
25   25% (w/v) sorbitol, 12 L; 60 g Pluronic P-123, 60 g
        Zonyl FSO, 1 L, sonicate to aid dissolution (stock
        surfactant solution). The Microfluidizer was primed
        with the sorbitol solution. The interaction chamber,
        tubing, and cooling coil are covered with chipped ice
30   during the comminution process. To a 5 L pressure
        vessel with stir bar in an ice bath add sequentially:
        500 mL sorbitol solution; 500 mL stock surfactant
        solution; 800 mL water; 200 g dodecafluoropentane.
        Pressurize vessel to 10 PSI with nitrogen for 45 min.

Pass the suspension through the Microfluidizer for 45
min at 14,000 PSI. Transfer the emulsion to a vessel
containing 8 L of 25% sorbitol at 4°C and mix well.
Transfer the emulsion to 100 mL vials using positive
5   pressure, passing the material through a 0.22 micron
filter in the process. Cap and seal the vials.

EXAMPLE 43
        A formulation of the present invention involves
the following equipment and steps: Microfluidizer,
10   Model 110Y, Interaction chamber pressure 14,000 PSI;
Pressure vessels, 316 steel, 5 L and 12 L sizes;
Filters, cellulose acetate, 0.22 micron; Filter
holders, 142 mm. The following solutions were made:
62.5% (w/v) sorbitol, 10 L; 41.75 g Pluronic P-123,
15   41.75 g Zonyl FSO, 2.5 L, sonicate to aid dissolution
(stock surfactant solution). The Microfluidizer was
primed with the sorbitol solution. The interaction
chamber, tubing, and cooling coil are covered with
chipped ice during the comminution process. To a 5 L
20   pressure vessel with stir bar in an ice bath add
sequentially: 1800 mL stock surfactant solution; 200
g dodecafluoropentane. Pressurize vessel to 10 PSI
with nitrogen for 45 min while stirring. Pass the
suspension through the Microfluidizer for 30 min at
25   5,000 PSI and for 60 min at 14,000 PSI. Transfer the
emulsion to a vessel containing 8 L of 62.5% sorbitol
at 4°C and mix well. Transfer the emulsion to 100 mL
vials using positive pressure, passing the material
through a 0.22 micron filter in the process. Cap and
30   seal the vials.

EXAMPLE 44

A formulation of the present invention involves
the following equipment and steps: Microfluidizer,
Model 110Y, Interaction chamber pressure 14,000 PSI;
5   Pressure vessels, 316 steel, 5 L and 12 L sizes;
Filters, cellulose acetate, 0.22 micron; Filter
holders, 142 mm. The following solutions were made:
33.3% (w/v) sucrose, 20 L; 150.0 g Pluronic P-123,
150.0 g Zonyl FSO, 2.5 L, sonicate to aid dissolution
10   (stock surfactant solution). The Microfluidizer was
primed with the sucrose solution. The interaction
chamber, tubing, and cooling coil are covered with
chipped ice during the comminution process. To a 5 L
pressure vessel with stir bar in an ice bath add
15   sequentially: 1800 mL stock surfactant solution; 333
g dodecafluoropentane. Pressurize vessel to 10 PSI
with nitrogen for 60 min while stirring. Pass the
suspension through the Microfluidizer at 14,000 PSI
for 160 min and with a circulating water bath cooling
20   the interaction chamber to -3.0°C. Transfer the
emulsion to a vessel containing 18 L of 33.3%, w/v
sucrose at 4°C and mix for 45 min. Transfer the
emulsion to 20 mL prechilled vials using positive
pressure, passing the material through a 0.22 micron
25   filter in the process. Cap and seal the vials.

EXAMPLE 45

The dispersed phase of the present invention
should be composed of any biocompatible chemical
having a boiling point at or below the body
30   temperature of the organism to which the formulation
is to be administered and which will be examined
following administration by ultrasound, such that a
sufficient quantity of the chemical becomes a gaseous
dispersion to provide a diagnostically useful
35   alteration in the ultrasound data obtained during the

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examination. Example 2 contains a table of the body
temperatures of a number of species which can be used
to select the appropriate dispersed phase for the
formulations disclosed herein.

5   Under certain conditions, for example, organisms
with febrile conditions or studies done in medical
facilities at high altitudes, where the air pressure
is lower, chemicals which have boiling points up to
18°C above the normal body temperature of the

10   organism could have utility as the dispersed phase
for such ultrasound contrast agents.

Having set the upper temperature limit for
selecting the dispersed phase low boiling liquid, the
lower limit is determined by the manufacturing

15   method. If the available equipment contains only
sealed vessels, and one cannot pressurize the
reaction vessel during the formulation of the
colloidal dispersion, only dispersed phases with
boiling points at or above the freezing temperature

20   of the continuous phase can be used. For example, a
continuous phase containing ca 25% w/v iohexol has a
freezing point near -6°C. Using such a continuous
phase, any low boiling liquid which boils above -6°C
can thus be liquified by cooling alone.

25   However if one can pressurize the reaction
vessel, for example with a nitrogen tank operating at
30 lb. per sq in. pressure, one can potentially
liquify and thus disperse any low boiling liquid,
even those boiling at temperatures below the freezing

30   point of the continuous phase.

Example 44 describes a method of forming an
emulsion with a dispersed phase liquid which boils
above the freezing point of the continuous phase,
while Example 48 below describes a method of forming

35   an emulsion by the application of both pressure and
refrigeration with a dispersed phase liquid which

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boils below the freezing point of the continuous
phase liquid. Obviously, any chemical will be more
efficiently dispersed by using some positive
pressure, to lower the vaporization of these
5   materials with the substantial vapor pressures that a
low boiling point implies.

Having determined the appropriate boiling point
of the dispersed phase liquid, the actual chemicals
which are useful can be quickly determined by
10   reference to standard texts, such as the CRC or a
similar compendium. A listing of some, but not all,
low boiling liquids arranged by boiling point
follows:

Chemical List: Boiling Points in degrees Celcius

Chemical Name	Molecular Weight	Boiling Point	Chemical Group
Neon	20.18	-246.0	11
Nitrogen (N2)	28.01	-196.0	11
Argon	39.98	-189.4	10
Oxygen (O2)	32	-183.0	11
Methane	16.04	-164.0	1
Krypton	83.8	-153.0	11
Nitric oxide	30.01	-151.6	11
Methane, tetrafluoro	88	-129.0	3
Xenon	131.29	-108.0	11
Ethylene	28.05	-103.7	1
Ethane	30.07	-88.6	1
Nitrous oxide	44.01	-88.5	11
Acetylene	26.04	-84.0	1
Methane, nitroso-trifluoro	99.01	-84.0	3
Methane, trifluoro	70.02	-84.0	3
Carbonyl fluoride	66.01	-83.0	9
Ethylene, 1,2-difluoro	64	-83.0	3
Ethylene, 1,1-difluoro	64.04	-83.0	3
Methane, trifluoro	70.01	-82.2	3
Methane, chloro trifluoro	104.46	-81.4	3
Ethane, hexafluoro	138.01	-79.0	3
Ethane, perfluoro	138.01	-79.0	3
Methane, fluoro	34.03	-79.0	3
Carbon dioxide	44.01	-78.6	11
Methane, fluoro	34.03	-78.4	3
Butyl nitrite	103.12	-77.8	11
Ethylene, tetrafluoro	100.02	-76.3	3
Sulfur hexafluoride	146.05	-64.0	11
Trifluoroacetonitrile	95.02	-64.0	10
Methane, bromo-trifluoro	148.91	-57.9	3
Methane, difluoro	52.02	-51.6	3
Ethylene, trifluoro	82.03	-51.0	3
Carbonyl sulfide	60.08	-50.0	11
Propyne, 3,3,3-trifluoro	94.04	-48.3	3
Ethane, Pentafluoro	120	-48.0	3
Propene	42.08	-47.4	1

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	Ethane, 1,1,1-trifluoro	84.04	-47.3	3
	Propane	44.1	-42.1	1
	Ethane, nitroso-pentafluoro	149.02	-42.0	3
	Methane, chloro-difluoro	86.47	-40.8	3
5	Propyl, 1,1,1,2,3,3-hexafluoro-2,3-difluoro	221	-39.03	3
	Allene, tetrafluoro	112.03	-38.0	3
	Ethane, 1-chloro-1,1,2,2,2-pentafluoro	154.47	-38.0	3
	Ethane, chloro pentafluoro	154.47	-38.0	3
	Ethane, fluoro	48.06	-37.7	3
10	Dimethylamine, perfluoro	171.02	-37.0	10
	Propane, perfluoro	188.02	-36.0	3
	Ethyl amine, perfluoro	171.02	-35.0	10
	Allene	40.06	-34.5	1
	Cyclopropane	42.08	-32.7	1
15	Trifluoromethyl peroxide	170.01	-32.0	11
	Azomethane, hexafluoro	166.03	-31.6	11
	Methane, nitro-trifluoro	115.01	-31.1	3
	Acetylene-chloro	60.48	-30.0	3
	Methane, dichloro difluoro	120.91	-29.8	3
20	Propylene, perfluoro	150.02	-29.4	3
	Acetone, hexafluoro	166.02	-28.0	3
	Ethane, 1,1,2,2-tetrafluoro	102.03	-27.0	3
	Ethane, 1,1,1,2-tetrafluoro	102.03	-26.5	3
	Ethylene, 1-chloro-1,2,2-trifluoro	116.47	-26.2	3
25	Ethylene, chloro trifluoro	116.47	-26.2	3
	Methyl ether	46.07	-25.0	6
	Ethane, 1,1-difluoro	66.05	-24.7	3
	2-Butyne, perfluoro	162.03	-24.6	3
	Ethylene, 1-chloro-1-fluoro	80.5	-24.0	3
30	Propyne	40.06	-23.2	1
	Methane, iodo-trifluoro	195.91	-22.5	3
	Trifluoromethyl sulfide	170.07	-22.2	11
	Methane sulfonyl fluoride, trifluoro	152.06	-21.7	3
	Propene, 3,3,3-trifluoro	96.05	-21.0	3
35	Propane, 1,1,1,3,3-Pentafluoro	132.04	-21.0	3
	Methane, (pentafluorothio)trifluoro	196.06	-20.0	3
	Ethane, 1,1,2,2-Tetrafluoro	102.04	-19.7	3
	Ethylene, 2-chloro-1, 1-difluoro	98.5	-17.7	3
	Propane, 2-H-heptafluoro	170.03	-15.0	3
40	Propane, 1,1,1-trifluoro	98.07	-13.0	3
	Methane, bromo difluoro nitroso	159.92	-12.0	3
	Methyl nitrite	61.04	-12.0	11
	Propane, heptafluoro-1-nitroso	199.03	-12.0	3
	Ethane, 2-chloro-1,1,1,2-tetrafluoro	136.48	-12.0	3
45	Isobutane	58.12	-11.6	1
	Ethane, 1-chloro-1,1,2,2-tetrafluoro	136.48	-10.0	3
	Propane, 2-fluoro	62.09	-10.0	3
	Methane, chloro fluoro	68.48	-9.1	3
	Isobutylene	56.11	-6.9	1
50	Dimethyl amine, hexafluoro	153.03	-6.7	10
	1-Butene	56.11	-6.3	1
	Nitrosyl chloride	65.47	-5.5	11
	1,3-Butadiene	54.09	-4.4	1
	Cyclobutane, octafluoro	200.03	-4.0	3
55	Propylene, 3-fluoro	60.07	-3.0	3
	Dimethyloxonium chloride	82.53	-2.0	3
	Propane, 2-chloroheptafluoro	204.47	-2.0	3
	Propane, 1,1,1,2,2,3-Hexafluoro	152.04	-1.4	3
	Propane, 1,1,1,3,3,3-Hexafluoro	152.05	-1.1	3
60	Methanesulfenylchloride, trifluoro	136.52	-0.7	3
	n-Butane	58.12	-0.5	1
	Propane, 2,2-difluoro	80.08	-0.4	3
	Ethane, 2-chloro, 1,1-difluoro	100	-0.1	3
	Ethane, nitro-pentafluoro	165.02	0.0	3
65	2-Butene, perfluoro	200.03	0.0	3

	Compound			
	Acetylene, isopropyl	68	0.0	1
	2-Butene (trans)	56.11	0.9	1
	1,2-Benzanthracene, 4-methyl	242.32	1.0	2
	Propane, 1,1,1,2,2,3-hexafluoro	152.04	1.2	3
5	2-Butene, octafluoro	200.04	1.2	3
	Azomethane	58.08	1.5	11
	Phthalic acid, tetrachloro	303.91	2.0	3
	Trimethyl amine	59.11	2.9	10
	Cyclobutene, perfluoro	162.03	3.0	3
10	1-Butene, 3,3,4,4,4-Pentafluoro	146	3.0	3
	Ethane, 1,2-dichloro-1,1,2,2-tetrafluoro	170.92	3.0	3
	Ethane, 1,1-dichloro-1,2,2,2-tetrafluoro	170.92	3.6	3
	2-Butene (cis)	56.11	3.7	1
	Ethane, 1,2-dichlorotetrafluoro	170.92	3.8	3
15	Butane, decafluoro	238.03	4.0	3
	Cyclopropane, methyl	56.11	4.0	1
	Ethane, dichlorotrifluoro	152	4.0	3
	Acetylene-bromo	104.93	4.7	3
	1-Butene, perfluoro	200.03	4.8	3
20	Benzoyl chloride, pentachloro	312.79	5.0	3
	Ethane, 1,1,2-trifluoro	84.04	5.0	3
	Vinyl acetylene	52.08	5.1	1
	1,3-Butadiene, hexafluoro	162.03	6.0	3
	Propene, 2-trifluoromethyl	110.08	6.0	3
25	Methanethiol	48.1	6.2	11
	Propane, 1,1,1,2,3,3-Hexafluoro	152.04	6.5	3
	Carbon suboxide	68.03	6.8	11
	Ethane, 2-chloro-1,1,1-trifluoro	118.49	6.9	3
	Fulvene	78.11	7.0	11
30	Dimethyl amine	45.08	7.4	10
	Propane, 2-chloro-1, 3-difluoro	114.51	8.0	3
	1-Butyne	54.09	8.1	1
	Methane, dichloro-fluoro	102.92	9.0	3
	Neopentane	72.15	9.5	1
35	Ethylene, 1-chloro-2-fluoro	80.5	10.0	3
	Butadiyne	50.06	10.3	1
	1,2-Butadiene	54.09	10.8	1
	Ethyl methyl ether	60.1	10.8	6
	1,3-Butadiene, 2-fluoro	72.08	12.0	3
40	Crotononitrile	67.09	12.0	11
	Cyclobutane	56.11	12.0	1
	Isobutane, 1,2-epoxy-3-chloro	106.55	12.0	3
	Methyl vinyl ether	58.08	12.0	6
	Propane, 1-bromo-heptafluoro	248.9	12.0	3
45	Ethane, idopentafluoro	245.9	12.0	3
	Propane, 2-(trifluoromethyl)-1,1,1,3,3,3-hexafluoro		211	12.03
	Ethane, Chloro	64.51	12.3	3
	Diazoethane, 1,1,1-trifluoro	110.04	13.0	3
	2-Butene, 3-methyl	68	14.0	1
50	Methane, disilano	76.25	14.7	11
	Ethyl nitrite	75.07	16.0	11
	Ethyl amine	45.08	16.6	10
	Tungsten hexafluoride	298	17.5	11
	2,3-Dimethyl-2-norbornano	140.23	19.0	11
55	Ethylene, 1,1-dichloro-2, 2-difluoro	133	19.0	3
	Methane, bromo fluoro	112.93	19.0	3
	1-Butene, 3-methyl	70.13	20.0	1
	Borine, trimethyl	55.91	20.0	11
	Fluorinert, FC-87 (3M Trade Mark)	Unknown	20.0	3
60	Cyclopropane, 1,1-dimethyl	70.13	20.6	1
	Acetaldehyde	44.05	20.8	7
	Acetyl flouride	62.04	20.8	9
	Borine, dimethyl, methoxy	71.19	21.0	11
	Ethylene, 1,2-dichloro-1,2-difluoro	132.92	21.1	3
65	Ethylene, dichloro difluoro	132.92	21.1	3

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Methane, difluoro-iodo	177.92	21.6	3
Diacetylene	50.08	22.0	1
Propylene, 2-chloro	76.53	22.6	3
Carvone- (d)	150.22	23.0	11
Methane, trichlorofluoro	137.37	23.7	3
1.3-Dioxolane-2-one, 4-methyl	102.09	24.2	1
Methane, dibromo difluoro	209.82	24.5	3
2-Pentanone, 4-amino-4-methyl	115.18	25.0	10
Methane, chloro difluoro nitro	131.47	25.0	3
Propane, heptafluoro-1-nitro	215.03	25.0	3
Cyclopentene, 3-chloro	102.56	25.0	3
1,4-Pentadiene	68.12	26.0	1
1,5-Heptadiyne	92.14	26.0	1
3-Butene-2-one, 4-phenyl (trans)	146.19	26.0	2
Propane, 1,1,2,2,3-Pentafluoro	134.06	26.0	3
2-Butyne	54.09	27.0	1
Ethane, 2,2-dichloro-1,1,1-trifluoro	152.9	27.0	3
Cyclopentene, Octafluoro	211.05	27.0	3
1-Nonene-3-yne	122.21	27.0	1
2-Methyl butane	72.15	27.8	1
Butane, 2-methyl	72.15	27.8	1
Ethane, 1,2-dichlorotrifluoro	152.9	28.0	3
Ether, difluoromethyl 2,2,2-trifluoroethyl	150.05	28.0	3
Cyclopropane, 1,2-dimethyl (trans, l)	70.13	28.0	1
Vinyl ether	70	28.0	6
Cyclopropane, 1,2-dimethyl (trans, dl)	70.13	29.0	1
Toluene, 2,4-diamino	122.17	29.0	2
1-Pentene, perfluoro	250.04	29.0	3
1-Butyne, 3-methyl	68.12	29.5	1
1-Pentene	70.13	30.0	1
1-Pentene, 3,3,4,4,5,5,5-heptafluoro	196	30.0	3
Ethylene, iodotrifluoro	207.9	30.0	3
Styrene, 3-fluoro	122.14	30.0	11
1-Pentene, 3-bromo	149.03	30.5	3
Pentane, perfluoro	288.04	30.5	3
Ethane, 1,2-difluoro	66.05	30.7	3
Butane, 3-methyl, 1,1,1-trifluoro	126.12	31.0	3
1-Butene, 2-methyl	70.13	31.2	1
Formic acid, methyl ester	60.05	31.5	9
Methane sulfonyl chloride, trifluoro	168.52	31.6	3
Ethane, 1,1-dichloro-1-fluoro	116.95	32.0	3
Pentane, 1-fluoro	90.14	32.0	3
Acetylene-diido	277.83	32.0	3
Propane, 2-amino	59.11	32.4	10
Butane, 1-fluoro	76.11	32.5	3
Methyl isopropyl ether	74.12	32.5	6
Propylene, 1-chloro	76.53	32.8	3
Butyraldehyde, 2-bromo	151	33.0	3
2-Butene, 2-chloro-1,1,1,4,4,4-hexafluoro	198.5	33.0	3
1,3-Butadiene, 1,2,3-trichloro	157.43	33.0	3
Butene, 2-chloro-1,1,1,4,4,4-hexafluoro	199	33.0	3
bis-(Dimethyl phosphino) amine	137.1	33.5	10
1,3-Butadiene, 2-methyl	68.12	34.0	1
1-Butene-3-yne, 2-methyl	66.1	34.0	1
Isoprene	68..12	34.0	1
Methane, chloro dinitro	140.48	34.0	3
Propane, 1,2-epoxy	58.08	34.3	6
Cyclopropane, ethyl	70.13	34.5	1
Ethyl ether	74.12	34.5	6
Dimethyl disulfide, hexafluoro	202.13	34.6	11
Ethylene, 1,2-dichloro-1-fluoro	115	35.0	3
Propane, 1,2-dichlorohexafluoro	220.93	35.0	3
Ethyl vinyl ether	72.11	35.0	6
Propane, 2-chloro	78.54	35.7	3
Methane, bromo-chloro-fluoro	147.37	36.0	3

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Piperidine, 2,3,6-trimethyl	127.23	36.0	11
1,2,3-Nonadecane tricarboxylic acid, 2-.....hydroxy, trimethylester	500.72	36.0	9
Dimethyl ethyl amine	73.14	36.0	10
n-Pentane	72.15	36.1	1
2-Pentene (trans)	70.13	36.3	1
Cyclobutane, methyl	70.13	36.3	1
Ethyl methyl amine	59.11	36.7	10
2-Pentene (cis)	70.13	36.9	1
Cyclopropane, 1,2-dimethyl (cis)	70.13	37.0	1
Ethylene, 1,1-dichloro	96.94	37.0	3
Propylene, 1-chloro-(trans)	76.53	37.4	3
Ethylene, 1,1-dichloro-2-fluoro	114.93	37.5	3
Methane, dichloro	84.93	40.0	3
Methane, iodo-	141.94	42.4	3
Ethane, 1,1-dichloro	98	57.3	3

CHEMICAL GROUP DESIGNATION
1 Aliphatic hydrocarbons and/or derivatives
2 Aromatic hydrocarbons and/or derivatives
3 Organic halides and/or derivatives
6 Ethers and/or derivatives
7 Aldehydes and/or derivatives
9 Carboxylic acids and/or derivatives
10 Amines and/of derivatives
11 Miscellaneous

EXAMPLE 46

The dispersed phase can also be selected from a group of azeotropes by the principles and criteria as set down in Example 45. A listing of some, but not all binary azeotropes, with the boiling points follows:

Acetone (21%)-Pentane (79%) 32°C; Ethyl ether (48%)-Isoprene (52%) 33°C; Ethyl ether (44%)-methyl formate (56%) 28°C; Ethyl ether (98.8%)-Water (1.2%) 34°C; Isoprene (86%)-2-methyl-2-butane (14%) 34°C; Isopropyl chloride (99%)-Water (1%) 35°C; Methyl vinyl chloride (99.1%)-Water (0.9%) 33°C; Pentane (98.6%)-Water (1.4%) 34°C; Vinyl ethyl ether (98.5%)-Water (1.5%) 34°C.

A listing of some but not all ternary azeotropes, with the boiling point follows:

Acetone (7.6%)-Isoprene (92%)-Water (0.4%) 32°C; Carbon disulfide -Methanol -Methyl acetate 37°C:

Carbon disulfide (55%)-Methanol (7%)-Methylal (38%)
35°C.

EXAMPLE 47

The colloidal dispersions of the present
invention are distinct and differ from prior art
emulsions for ultrasound contrast in that at least
some portion of the dispersed phase percolates or
vaporizes following administration to an organism.
The presence of this dispersed material with a
distinct liquid-gas interface provides the basis for
the strong backscatter of the acoustic beam.

One test of the presence of a dispersed gas
phase emulsion is the response of the ultrasound
backscatter from the dispersion to changes in
pressure. While true liquid dispersions are largely
insensitive to compressive forces, a gaseous
colloidal dispersion will show a decrease in acoustic
backscatter when pressure is applied, due to
compression of the gas and a decrease in the
effective backscatter cross section.

With the experimental system of Example 1, the
acoustic backscatter in a sealed beaker was tested
through an acoustic window. Then pressure was
applied to the system and rerecording the acoustic
backscatter recorded. Since the acoustic backscatter
differed significantly following the application of
pressure it was concluded that the dispersed phase
contains some portion in the gas state.

EXAMPLE 48

A formulation of the present invention can be
made by condensation of the dispersed phase from the
gas state rather than comminution from the liquid
state and involves the following equipment and steps:
Microfluidizer, Model 110Y, Interaction chamber

pressure 14,000 PSI; Pressure vessels, 316 steel, 5 L
and 12 L sizes; Filters, cellulose acetate, 0.22
micron; Filter holders, 142 mm. The following
solutions were made: 36% iohexol, 10 L; 41.75 g
5   Pluronic P-123, 41.75 g Zonyl FSO, 2.5 L, sonicate to
aid dissolution (stock surfactant solution). The
Microfluidizer was primed with the iohexol solution
and the entire container cooled to -6°C. The
interaction chamber, tubing, and cooling coil are
10   covered with chipped ice during the condensation
process. To a 5 L pressure vessel with stir bar in
an ice bath add 1800 mL stock surfactant solution. A
tank of propane (boiling point -42°C) was attached to
the interaction chamber by gas tight fittings and the
15   chamber charged with 200 g of propane. The entire
vessel was pressurized to 10 PSI with nitrogen for 45
min while stirring. The suspension was passed
through the Microfluidizer for 30 min at 5,000 PSI
for 60 min at 14,000 PSI. The emulsion was
20   transferred to a vessel containing 8 L of water at
4°C and mixed well and transferred to 100 mL vials
using positive pressure, passing the material through
a 0.22 micron filter in the process. Cap and seal
the vials.

25   Other emulsions containing other low boiling
materials of Example 45 can be made in a similar
manner by varying the dispersed phase and being
certain the pressure and temperature are sufficient
to liquify the dispersed phase material.

30   EXAMPLE 49
           The dispersed phase can be composed of any
chemical which has a boiling point under standard
pressure conditions below the body temperature of the
organism to which the formulation is to be
35   administered and which will be examined following

administration by ultrasound. Example 45 discusses
how one selects suitable chemicals for the dispersed
phase based on the temperature range obtained by
consideration of the boiling point of the selected
5   chemical and parameters of the manufacturing process.
    Having determined that the boiling point under
standard conditions of pressure is preferably below
approximately 37°C, it has been found that selecting
chemicals by the total number of atoms present
10   provides an alternative method of selecting suitable
materials as ultrasound contrast agents. A listing
of suitable chemicals, arranged by total atoms
present, reveals that all preferred chemicals contain
between four and seventeen atoms and follows:

Chemical List: Boiling Points in degrees Celcius

	Name	Total Atoms	Molecular Formula	Molecular Weight	Boiling Point
	bromo-methane	4	CH3Br	94.94	3.2
	bromo-difluoro-methane	5	CHBrF2	130.92	-14.15
	chloro-fluoro-methane	5	CH2ClF	68.48	-9.15
	bromo-trideuterio-methane	5	CD3Br	12	2.8
	propadienedione	5	C3O2	68.03	6.8
	dicholoro-fluoro-methane	5	CHCl2F	102.92	8.9
	methaneselenol	5	CH4Se	95	12
	difluoro-iodo-methane	5	CHF2I	177.92	21.6
	dibromo-difluoro-methane	5	CBr2F2	209.82	22.79
	trichloro-fluoro-methane	5	CCl3F	137.7	23.65
	bromo-chloro-fluoro-methane	5	CHBrClF	147.37	36.11
2-chloro- 1,1-difluoro-ethene	6	C2HClF2	98.48	-18.6
	trifluoro-methaneselenol	6	CHF3Se	148.97	-14.5
	chloro-ethene	6	C2H3Cl	62.5	-13.9
	oxalyl fluoride	6	C2F2O2	94.02	-2.7
	formamide	6	CH3NO	45.04	2.18
2-bromo- 1,1-difluoro-ethene	6	C2HBrF2	142.93	5.7
	methanethiol	6	CH4S	48.1	5.9
	butadiyne	6	C4H2	50.06	9
	bromo-ethene	6	C2H3Br	106.95	15.6
1,1- dichloro-2,2-difluoro-ethene	6	C2Cl2F2	132.92	18.9
trans-1- bromo-2-fluoro-ethylene	6	C2H2BrF	124.94	19.8
	bromo-methane	4	CH3Br	94.94	3.2
1,1- dichloro-2,2-difluoro-ethane	6	C2Cl2F2	132.92	20
1,1 dichloro-ethene	6	C2H2Cl2	96.94	31.8
trans-1,2 Dichlorfluoroethylene	6	C2HCl2F	114.93	37
cis Dichlorofluoroethylene	6	C2HCl2F	114.93	37
1,1 dichloro-2-fluoro-ethene	6	C2HCl2F	114.93	37
Methyldifluoroamine	7	CH3F2N	67.02	-16
difluorophosphoric acid methyl ester	7	CH3F2OP	100	-14.5
methylamine	7	CH5N	31.06	-6.5
dichloro-methyl-borane	7	CH3BCl2	96.75	11.5
tetrachloro-1,2-difluoro-ethane	8	C2Cl4F2	203.83	-37.5
1,1,2- trichloro-ethane	8	C2H3Cl3	133.4	-24
1,1,1,2- tetrachloro-ethane	8	C2H2Cl4	167.85	-16.3
1-chloro- 1,1-difluoro-ethane	8	C2H3ClF2	100.5	-9.8
1,2- dibromo-1,1-dichloro-ethane	8	C2H2Br2Cl2	256.75	1.78
1,1- dichloro-tetrafluoro-ethane	8	C2Cl2F4	170.92	3
1,1,2- trifluoro-ethane	8	C2H3F3	84.04	3
1,2- dichloro-tetrafluoro-ethane	8	C2Cl2F4	170.92	3.5
Tetrafluor-(methyl-methylamine)	8	C2HF4N	115.03	5
	butenyne	8	C4H4	52.08	5.11
2-chloro- 1,1,1-trifluoro-ethane	8	C2H2ClF3	118.49	6
Fluorcarbonyl-trifluormethyl-sulfane	8	C2F4OS	148.08	8
chloro-methyl-silane	8	CH5ClSi	80.59	8
1,2- difluoro-ethane	8	C2H4F2	66.05	10
chloro-ethane	8	C2D5Cl	64.51	12
pentafluoro-iodo-ethane	8	C2F5I	245.92	12.5
2- diazo-1,1,1-trifluoro-ethane	8	C2HF3N2	110.04	13
1-chloro- 1-fluoro-ethane	8	C2H4ClF	82.31	16
1,1,2- tetrachloro-ethane	8	C2H2Cl4	167.85	16.3
1,1,2- trichloro-ethane	8	C2H3Cl3	133.4	24
2- bromo-1,1,1-trifluoro-ethan	8	C2H2BrF3	162.94	26
Chlormethyl-trifluor-silane	8	CH2ClF3Si	134.56	26
1,2- difluoro-ethane	8	C2H4F2	66.05	30.7
2- chloro-1,1-difluoro-ethane	8	C2H3ClF2	100.05	35.1
tetrachloro-1,2-difluoro-ethane	8	C2Cl4F2	203.83	37.5
	bromo-pentadeuterio-ethane	8	C2BrD5	114	38

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		dimethyl-silane	9	C2H8SI	60.1	-20
		Pentafluor-cyclopropane	9	C3HF5	132.03	-9
		difluoromethyl-trifluoromethyl sulfide	9	C2HF5S	152.08	0.8
	1,1,2,3,3-	pentafluoro-propene	9	C3HF5	132.03	1.8
5		Chlorpentafluorcyclopropane	9	C3ClF5	166.48	3
		Germylacetylene	9	C2H4Ge	100.64	3.85
	rans-1,1,2,3	Tetrafluorcyclopropane	9	C3H2F4	114.04	6
	2-	chloro-pentafluoro-propene	9	C3ClF5	166.48	6.7
	3-	chloro-pentafluoro-propene	9	C3ClF5	166.48	7.3
10	1-	chloro-pentafluoro-propene	9	C3ClF5	166.48	8
		fluoro-methyl-methyl ether	9	C2H5FO	64.06	19
		Brompentafluorcyclopropane	9	C3BrF5	210.93	20.5
		Vinyloxy-acetylene	9	C4H4O	68.08	22
	2-	chloro-propene	9	C3H5Cl	76.53	22.6
15	cis-trans-1-	Chlor-1,2,2,3-tetrafluorcyclopropane	9	C3HClF4	148.49	24.5
	3-	bromo-pentafluoro-propene	9	C3BrF5	210.93	26.5
	2,2-	dichloro-1,1,1-trifluoro-ethane	9	C2HCl3F3	152.93	27
		Furan	9	C4H4O	68.08	31
	1-	chloro-propene	9	C3H5Cl	76.53	32.1
20	2-	Chlor-vinyl-trifluorsilane	9	C2H2ClF3SI	146.57	33
	cis-1,1,2,3	Tetrafluorcyclopropane	9	C3H2F4	114.04	34
	3-	bromo-1,1,3,-tetrafluoro-propene	9	C3HBrF4	192.94	34
		ethanethiol	9	C2H6SI	62.13	35
		dimethyl sulfide	9	C2H6S	62.13	36
25	(| chloro-fluoro-methyl)-trifluoromethyl sulfide	9	C2HClF4S	168.54	37
	1 t-	chloro-propane	9	C3H5Cl	76.53	37.2
		buta-1,3-diene	10	C4H6	54.09	-4.6
	1,5-	Dicarbaclosotriborane	10	C2H5B3	61.49	-3.7
30	omega-	Nitrosoperfluorpropionnitrile	10	C3F4N2O	156.04	2
		pentafluoro-propionaldehyde	10	C3HF5O	148.03	2
	1,1-	difluoro-buta-1,3-diene	10	C4H4F2	90.07	3.5
		methyl-vinyl ether	10	C3H6O	58.08	5
		hexafluoro-buta-1,3-diene	10	C4F6	162.03	5.8
35		but-1-yne	10	C4H6	54.09	7.9
		1-Deutero-1-butane	10	C4H5D	55.1	8
		methylene-cyclopropane	10	C4H6	54.09	8.8
		buta-1,2-diene	10	C4H6	54.09	10.84
40	2-	fluoro-buta-1,3-diene	10	C4H5F	72.08	11.5
	1H-	pentafluoro-but-1-yne	10	C4HF5	144.04	12
		pentafluoro-acetone	10	C3HF5O	148.03	13.5
		Difluoraminoethane	10	C2H5F2N	81.07	14.9
		Tetra-B-fluor-B,B'-ethenodiyl-bis-borane	10	C2H2B2F4	123.65	15
45	cis-1-	Fluor-1,3-butadiene	10	C4H5F	72.08	15.6
	trans-1-	Floor-1,3-butadiene	10	C4H5F	72.08	16
		ethylamine	10	C2H7N	45.08	16.6
		dimethyl-phosphine	10	C2H7P	62.05	20
	N-	Methyl-imino-schwefel-tetra-fluoride	10	CH3F4NS	137.1	21.8
50		Methylschwefelpentafluoride	10	CH3F5S	142.09	26
		but-2-yne	10	C4H6	54.09	26.97
		bromo-pentafluoro-acetone	10	C3BrF5O	226.93	31
		bromo-dimethyl-borane	10	C2H6BBr	120.78	31.75
	1-	chloro-2,3,3,4,4-pentafluoro-cyclobutene	10	C4ClF5	178.49	33
55	bis-	trifluoromethyl disulfide	10	C2F6S2	202.13	34
	(±)(1)1,2-	epoxy-propane	10	C3H6O	58.08	34.23
		ethyl-silane	11	C2H8SI	60.17	-14
	1,1,1-	trifluoro propane	11	C3H5F3	98.07	-13
60	2-	fluoro-propane	11	C3H7F	62.09	-11
		Perfluormethoxyacetylfluoride	11	C3F6O2	182.02	-9.7
		ethyl-trifluoro-silane	11	C2H5F3SI	114.14	-4.4
	1-	fluoro-propane	11	C3H7F	62.09	-3
	2,2-	difluoro-propane	11	C3H6F2	80.08	-0.6
65	1,1,1,3,3,3-	hexafluoro-propane	11	C3H2F6	152.04	-0.5

Perfluorcyclobutanone	11	C4F6O	178.03	1
1,1,1,2,2,3-hexafluoro-propane	11	C3H2F6	152.04	1.2
2- chloro-heptafluoro-propane	11	C3ClF7	204.47	2.2
dideuterio-dimethyl germane	11	C2H6D2Ge	106.69	6.5
1,1- difluoro-propane	11	C3G6F2	80.08	7
ethyl-trideuterio germane	11	C2H5D3Ge	107.69	11.3
disilanyl-methane	11	CH8SI2	76.25	14
1-chloro- 1,1,2,2-tetrafluoro-propane	11	C3H3ClF4	150.5	19.93
Trifluorosilydimethylamine	11	C2H6F3NSi	129.16	21
ethylidene-methyl-amine	11	C3H7N	57.1	27.5
disilanyl-methane	11	CH8SI2	76.25	28
divinyl ether	11	C4H6O	70.09	28
1,1,1,3- tetrafluoro-propane	11	C3H4F4	116.06	29.4
1- Sila-3-germapropane	11	CH8GeSI	120.75	30
2- chloro-1,1,1-trifluoro-propane	11	C3H4ClF3	132.51	30
2- methyl-but-1-en-3-yne	11	C5H6	66.1	32
Bis-trifluorsilyldichlormethane	11	CCl2F6SI2	253.08	34
1,2- dichloro-hexafluoro-propane	11	C3Cl2F6	220.93	34.8
2- chloro-propane	11	C3H7Cl	78.54	34.8
ethyl-vinyl ether	11	C2H8O	72.11	35
3- methylen-oxetane	11	C4H6O	70.09	35
2- chloro-2-fluoro-propane	11	C3H6ClF	96.53	35.2
Bis- trifluorsilylmethan	11	CH2F6SI2	184.19	35.5
chloro-dimethyl-silane	11	C2H7ClSi	94.62	35.7
1,3 dichloro-hexafluoro-propane	11	C3Cl2F6	220.93	36.1
Bis-trifluorsilylchlormethane	11	CHClF6SI2	218.63	37
heptafluoro-1-nitroso-propane	12	C3F7NO	199.03	-9.5
1,1,2,2,3- pentafluoro-3-trifluoromethyl-cyclopropane	12	C4F8	200.03	-9
2- methyl-propene	12	C4H8	56.11	-6.9
octafluoro-cyclobutane	12	C4F8	200.03	-6.42
but-1-ene	12	C4H8	56.11	-6.3
1,1,2,2- Tetrafluor-2-trifluormethoxy-aethane	12	C3HF7O	186.03	-2
cis-octafluoro-butene-(2)	12	C4F8	200.03	0.4
methyl-cyclopropane	12	C4H8	56.11	0.7
but-2 t-ene	12	C4H8	56.11	0.88
butene-(2)	12	C4H8	56.11	1
heptafluoro-butyronitrile	12	C4F7N	195.04	1
octafluoro-but-2-ene	12	C4F8	200.03	1.2
1,1- difluoro-but-1-ene	12	C4H6F2	92.09	3.71
but-2 c-ene	12	C4H8	56.11	3.72
octafluoro-but-1-ene	12	C4F8	200.03	4.8
1,1,1,4,4,4-Hexafluor-2-butene	12	C4H2F6	164.05	5.4
trifluormethylethylether	12	C3H5F3O	114.07	5.5
2H,3H- hexafluoro-but-2 t-ene	12	C4H2F6	164.05	6
3,3,3- trifluoro-2-methyl-propene	12	C4H5F3	110.08	6
ethyl-methyl ether	12	C3H8O	60.1	6.6
2H- Heptafluor-buten-(1)	12	C4HF7	182.04	10
cyclobutane	12	C4H8	56.11	12
pentafluoro-2-methyl-propene	12	C4H3F5	146.06	12.8
Methyl-vinylsilane	12	C3H8Si	72.18	13.7
1,1,1- trifluoro-but-2 t-ene	12	C4H5F3	110.08	20
1,1,1- trifluoro-but-2 t-ene	12	C4H5F3	110.08	20
Allyltrifluorsilane	12	C3H5F3Si	126.15	21
1,1,2 Trifluor-2-trifluormethylcyclopropane	12	C4H2F6	164.05	21.5
1,1,2- Trifluor-1-chlor-2-trifluormethoxy-aethane	12	C3HClF6O	202.48	23
heptafluoro-propane-1-thiol	12	C3HF7S	202.9	23.7
(2- Brom-1,1,2,2-tetrafluor-ethyl)-trifluormethyl-ether	12	C3BrF7O	264.93	24
Cyclopropylsilane	12	C3H8Si	72.18	26.8
3,3 difluoro-2-methyl-propene	12	C4H6F2	92.09	28.1
1,1,2,2- Tetrafluorethyldifluormethylether	12	C3H2F6O	168.04	28.5
2,2,2 Trifluorethyl-difluormethylether	12	C3H3F5O	150.05	29

1,1,1-	trifluoro-2-methoxy-ethane	12	C3H5F3O	114.07	31
2-	chloro-heptafluoro-but-2-ene	12	C4ClF7	216.49	32.2
	Pentafluoronitrosoacetone	12	C3F5NO3	193.03	32.6
2H,3H	hexafluoro-but-2 c-ene	12	C4H2F6	164.05	33.2
2-	chloro-3H-hexafluoro-but-2-ene	12	C4HClF6	198.5	34.4
	tetra-B-fluoro-B,B'-ethanediyl-bis-borane	12	C2H4B2F4	125.67	35
	Ethyl-trifluormethyl-sulfide	12	C3H5F3S	130.13	35
	methyl-(1,1,2,2-tetrafluoro-ethyl)-ether	12	C3H4F4O	132.06	36.5
(| Chlor-difluormethyl)-(2,2,2-trifluor-ethyl)-ether	12	C3H2ClF5O	184.49	37
1,1,2-	Trifluor-1,2-dichlor-2-trifluormethoxy-aethane	12	C3Cl2F6O	236.93	37
1-	Nitroso-2-trifluormethoxy-tetrafluorethane	13	C3F7NO2	215.03	-10
	Nonafluor-2-azabutane	13	C3F9N	221.03	-3.8
	trimethyl-amine	13	C3H9N	59.11	3.5
3,3-	Dimethyl-cyclopropene	13	C5H8	68.12	18
	penta-1,4-diene	13	C5H8	68.12	24
3-	methyl-but-1-yne	13	C5H8	68.12	26
3-	Methyl-cyclobutene	13	C5H8	68.12	27.5
	Trifluormethanazo-2,2,2-trifluoraethane	13	C3H2F6N2	180.05	28
2-	methyl-buta-1,3-diene	13	C5H8	68.12	30
	alpha-Nitroso-perfluorisobutyronitrile	13	C4F6N2O	206.05	31
	isopropylamine	13	C3H9N	59.11	31.7
2-	Methoxyperfluoropropene	13	C4H3F5O	162.06	32
	Dimethylethinylsilane	13	C4H8Si	84.19	32
1,1,2,2-	Tetradeuterospiropentane	13	C5H4D4	72.14	33
	dimethoxy-silane	13	C2H8O2Si	92.17	33.5
	isopropenyl-methyl ether	13	C4H8O	72.11	34
	tert-butyl-silane	13	C4H12Si	88.22	34.4
	spiropentane	13	C5H8	68.12	35
3,4,4-	Trifluorisoprene	13	C5H5F3	122.09	35
1-	methyl-cyclobutene	13	C5H8	68.12	37
2-	methyl-propane	14	C4H10	58.12	-13.3
	decafluoro-butane	14	C4F10	238.03	-1.7
1-	deuterio-butane	14	C4H9D	59.13	-0.5
	butane	14	C4H10		-0.5
	Perfluorethoxyacetylfluoride	14	C4F8O2	232.3	0
	trimethyl-silane	14	C3H10Si	74.2	6.7
	Trifluormethylpentafluor-2-oxapropylketone	14	C4F8O2	232.03	8
2-fluoro-	2-methyl-propane	14	C4H9F	76.11	11
	pentafluoroethyl-tetrafluoroethyliden-amine	14	C4F9N	233.04	12.8
2-	Trifluoromethyl-propane	14	C4H7F3	112.09	13
	Perfluor-2-aza-penten-(2)	14	C4F9N	233.04	13.2
	fluoro-trimethyl-silane	14	C3H9FSi	92.19	16
1,1,1-	trifluoro-butane	14	C4H7F3	112.09	16.74
	dimethyl-vinyl-borane	14	C4H9B	67.93	17.1
	Tris-(trifluormethyl)-germaniumfluoride	14	C3F10Ge	298.61	19.1
	fluoro-trimethyl-silane	14	C3H9FSi	92.19	20
	propyl-silane	14	C3H10Si	74.2	21.3
1,1,1,3,3,3-hexafluoro-2-methyl-propane		14	C4H4F6	166.07	21.5
2-	fluoro-butane	14	C4H9FF	76.11	24
1,1,1,4,4,4-hexafluoro-butane		14	C4H4F6	166.07	24.5
	methoxy-dimethyl-borane	14	C3H9BO	71.91	24.6
	trifluoro-propyl-silane	14	C3H7F3Si	128.17	25
	Deuterio-trimethyl germane	14	C3H9DGe	119.71	26
	Trimethyl Germane	14	C3H10Ge	118.7	26
	trimethyl-hydroxylamine	14	C3H9NO	75.11	30
2,2	difluoro-butane	14	C4H8F2	94.1	30.92

1- fluoro-butane	14	C4H9F	76.11	31
Tris-. (trifluoromethyl)-germaniumchloride	14	C3ClF9Ge	315.06	37
nonafluoro-1-nitroso-butane	15	C4F9NO	249.04	16
3- methyl-but-1-ene	15	C5H10	70.13	20
1,1- dimethyl-cyclopropane	15	C5H10	70.13	20
3- methyl-but-1-ene	15	C5H10	70.13	20
1,1- dimethyl-cyclopropane	15	C5H10	70.13	20.6
decafluoro-cyclopentane	15	C5F10	250.04	22.48
1,1,1,3,3,3-Hexafluor-2-nitroso-2-trifluormethyl-propane	15	C4F9NO	249.04	24
± Trans-1,2-dimethyl-cyclopropane	15	C5H10	70.13	28.2
1,2 dimethyl-cyclopropane	15	C5H10	70.13	28.8
pent-1-ene	15	C5H10	70.13	29
1- Nitroso-4-monohydrooctafluorbutane	15	C4HF8NO	231.05	30
trifluoro-acetic acid-(bis-trifluoromethyl-amide)	15	C4F9NO	249.04	30
isopropyl-methyl ether	15	C4H10O	74.12	30.77
2- methyl-but-1-ene	15	C5H10	70.13	30.95
Perfluorpropylmethylether	15	C4H3F7O	200.06	34
diethyl ether	15	C4H10O	74.12	34.6
ethyl-cyclopropane	15	C5H10	70.13	35.8
methyl-cyclobutane	15	C5H10	70.13	36
pent-2-ene	15	C5H10	70.13	36.15
pent-2 c-ene	15	C5H10	70.13	36.55
cis-1,2- dimethyl-cyclopropane	15	C5H10	70.13	37.03
2- methyl-but-2-ene	15	C5H10	70.13	37.2
beta- Nitroso-nonafluordiethylether	16	C4F9NO2	265.04	15
nitrous acid ethyl ester	16	C2H5NO2	75.07	17.4
Perfluor-diethylamine	16	C4F11N	271.03	23.9
Perfluor-2-aza-pentan	16	C4F11N	271.03	24.3
4- Methylpent-4-enacurenitrile	16	C6H9N	95.14	30
butyl-difluoro-borane	16	C4H9BF2	105.92	35
ethyl-dimethyl-amine	16	C4H11N	73.14	36.4
3,3 dimethyl-but-1-yne	16	C6H10	82.15	37
2,2- dimethyl-propane	17	C5H12	72.15	0.95
(-)(S)-1- fluoro-2-methyl-butane	17	C5H11F	90.14	14.1
(-)(R)-2- chloro-pentane	17	C5H11Cl	106.59	24.7
Tetramethyl-stannane	17	C4H12Sn	178.83	26
2- methyl-butane	17	C5H12	72.15	27.85
nonafluoro-2-trifluoromethyl-butane	17	C5F12	288.04	30.12
Tetrakis(trifluoromethyl) germane	17	C4F12Ge	348.61	31.7
pentane	17	C5H12	72.15	36

EXAMPLE 50

In a preferred embodiment, the dispersed phase can be composed of any chemical which has a boiling point under standard pressure conditions below the body temperature of the organism to which the formulation is to be administered and which will be examined following administration by ultrasound. Example 45 discusses how one selects suitable chemicals for the dispersed phase based on the temperature range obtained by consideration of the boiling point of the selected chemical and parameters of the manufacturing process.

The boiling of pentane (dodecahydropentane) and
perfluoropentane (Dodecafluoropentane) are 36-37°C
and 28-29°C, respectively. This is an excellent
temperature range in which to select suitable
chemicals as the dispersed phase. Therefore,
chemicals which contain five carbon atoms and
variable hydrogen and fluorine atoms will have
boiling points between 28 and 37°C and will make
suitable dispersed phase chemicals. The following
listing of suitable chemicals contains some, but not
all chemicals containing five carbons, with variable
numbers of hydrogen and fluorine atoms, i.e., $C_5H_xF_y$:

1,3-Cyclopentadiene, 5,5-difluoro-;Cyclobutane,
1-fluoro-3-methylene-;2H-Fluorinium;Cyclobutane, (fluoromethylene)-;Methylene,
cyclobutylfluoro-;2,4-Cyclopentadien-1-yl, 2-fluoro-;2H-Fluorinium, ion(-1),
(deloc-2,3,4,5,6)-;6-Fluoroniabicyclo(3.1.0)hexane;6-Fluoroniabicyclo(3.1.0)hex-2-ene,
hydroxide, inner salt;Fluorine(2+),1,3-pentadien-1-yl-5-ylidene-;1,3-Pentadiene, fluorine
complex;Fluoranium;Cyclopentyne, 4-fluoro-;Cyclobutene, 3-(trifluoromethyl)-;Cyclopentane,
1,1,2,2,3,3-hexafluoro-;Tricyclo(1.1.1.01,3)pentane, fluoro-, ion(1-);Spiro(2.2)pentane,
fluoro-, ion(-1);Tricyclo(1.1.1.01.3)pentane, fluoro-;cyclopentane, 1,2-difluoro-,
trans-;Cyclobutane, 1,1-difluoro-3-methylene;1,3-Cyclopentadiene,
2-fluoro-;1,3-Cyclopentadiene, 1-fluoro-;Bicyclo(1.1.1)pentane,
1,3-difluoro-;1,3-Cyclopentadiene, 1,2,3,4,5-pentafluoro-, dimer;1,3-Cyclopentadiene,
1,2,3,4-tetrafluoro;1,3-Cyclopentadiene, 1,2,3,4,5-pentafluoro-;Cyclopentene,
1,2,3,3,4,5-hexafluoro-;Cyclobutane, 1,1,2,2,3-pentafluoro-3-(trifluoromethy)-;Cyclobutene,
3,3,4,4-tetrafluoro-1-methyl-;Cyclobutane, 1-fluoro-1-methyl-;Bicyclo(2.1.0)pentane,
2,2,3,3-tetrafluoro-;Cyclopentene, 3,3-difluoro-;1,3-Cyclopentadiene, 5-fluoro-;Cyclobutane,
2-(difluoromethylene)-1,1,3,3-tetrafluoro-;Spiro(2.2)pentane,
1,1,2,2,4,4-hexafluoro-;Bicyclo(1.1.1)pentane, 1-fluoro-;Cyclopentene,
4,4-difluoro-;Cyclobutane, (difluoromethylene)-;Cyclobutane,
1,1-difluoro-2-methylene-;Spiro(2.2)pentane, 1,1-difluoro-;Cyclobutane,
1,1,3,3-tetrafluoro-2-methylene-;Cyclobutane,
2-(difluoromethylene)-1,1-difluoro-,Spiro(2.2)pentane, 1,1,4,4-tetrafluoro-;Cyclopropane,
1,1-bis(trifluoromethyl)-;Spiro(2.2)pentane, 1,1,2,2-tetrafluoro-;Tricyclo(1.1.0.02.4)butane,
(trifluoromethyl)-;Spiro(2.2)pentane, 1,4-difluoro-;Spiro(2.2)pentane,

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1,2-difluoro-;Spiro(2.2)pentane, fluoro-;Bicyclo(1.1.0)butane,

1-(trifluoromethyl)-;Cyclopentane, 1,2-difluoro-, cis-;Cyclopropane,

(1,1,2-trifluoroethyl)-;Cyclopropane, (1,1-difluoroethyl)-;Cyclopropane,

(1,2,2-trifluoroethyl)-;Cyclopropane, (2,2-difluoroethyl)-;Cyclopropane,

5   (2-fluoroethyl)-;Cyclopropyl, 1-fluoro-2, 2dimethyl-;Cyclopropyl, 1-fluoro-2, 3-dimethyl-,

cis-;Cyclobutane, (trifluoromethyl)-;Fluoriranium, trimethyl-;Cyclopentylium,

1-fluoro-;Cyclopropane, 1,1-difluoro-2-methyl-2-(trifluoromethyl)-;Cyclopropane,

1-fluoro-2,3-dimethyl-,(1.alpha.,2.alpha.,3.alpha.)-;Cyclopropane,

1-fluoro-2,3-dimethyl-,(1.alpha.,2.beta.,3.beta.)-;Cyclopropane,

10   1-ethyl-2-fluoro-;Cyclopropane, 1-ethyl-2-fluoro-, trans-;Cyclopropane,

1-fluoro-2,3-dimethyl-,(1.alpha.,2.alpha.,3.beta.)-;Cyclobutane,

1,1,2-trifluoro-2-(trifluoromethyl)-;Cyclopropane, 1-(difluoromethyl)-1-fluoro-2-methyl-,

trans-;Cyclopropane, 1-(difluoromethyl)-1-fluoro-2-methyl-,cis-;Cyclobutane,

1,1,2,2,3-pentafluoro-3-methyl-;Cyclobutane,

15   1,1,2,3-tetrafluoro-2-(trifluoromethyl)-;Cyclopropane, (2-fluoroethenyl)-;Cyclopropane,

(1-fluoroethenyl)-;Bicyclo(2.1.0)pentane, 5,5-difluoro-;Cyclobutene,

1,4,4-trifluoro-3-methylene-;Cyclopropane, 2-etheynyl-1, 1-difluoro-,

homopolymer;Cyclobutane, 3-(difluoromethylene)-1,1-difluoro-;Cyclopropane,

1,1,2-trifluoro-2-(trifluorovinyl)-;Cyclopentene, 1-fluoro-;Cyclopropane, 2-ethyl-1,

20   1-difluoro-;Cyclopropene, 3,3-difluoro-1-(pentafluoroethyl)-;Cyclopropane,

1-methyl-2-(trifluoromethyl)-, cis-;Cyclopropane, 1-methyl-2-(trifluormethyl)-,

trans-;Cyclopropane, 1-methylene-2-(trifluoromethyl)-;Cyclopentane,

1,1,2,2,3,3,4,5-octafluoro-;Cyclopropane, 1-(difluoromethyl)-1-fluoro-2-methyl-,

cis-;Cyclopropane, 1-(difluoromethyl)-1-fluoro-2-methyl-, trans-;Cyclopentane.

25   1,1,2,2,3,3,4-heptafluoro-;1,3-Cyclopentadiene, 1,2,4,5,5-pentafluoro-,

dimer;1,3-Cyclopentadiene, 1,2,3,5,5-pentafluoro-, dimer;1,3-Cyclopentadiene,

1,2,3,5,5-pentafluoro-;1,3-Cyclopentadiene, 1,2,4,5,5-pentafluoro-;Cyclopentane,

1,2,3,4,5-pentafluoro-,stereoisomer;Cyclopentane,

1,1,2,3,4,5-hexafluoro-,stereoisomer;Cyclobutene, 3-fluoro-1-methyl-;Cyclopentene,

30   1,4,5,5-tetrafluoro-;Cyclopentene, 3,3,4,4-tetrafluoro-;Cyclopentene,

3,3,4,4,5-pentafluoro-;Cyclopentene, 1,4,4,5,5-pentafluoro-;Cyclopentene,

1,3,3,4,4,5-hexafluoro-;Cyclopropane, (2,2,2-trifluoroethyl)-;Cyclopentane,

1,1,2,3,3,4,5-heptafluoro-;Cyclobutene, 2,3,3-trifluoro-1-(trifluoromethyl)-;Cyclopentene,

1,2,3,3,4,5,5-heptafluoro-;Cyclopentene, 1,2,3,3,4,4,5-heptafluoro-;Cyclobutene.

35   3,3,4,4-tetrafluoro-1-(trifluoromethyl)-;Cyclopentene, 1,3,3,4,4,5,5-heptafluoro-;Cyclopropane,

2-fluoro-1, 1-dimethyl-;Cyclopentane, 1,1,2,2,3,4,5-heptafluoro-;Cyclobutane,

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1,1,2,2-tetrafluoro-3-(trifluoromethyl)-;Cyclopentane, fluoro-;Cyclopentene,

1,2,3,3,4,5-hexafluoro-, trans-;Cyclopentane, 1,1-difluoro-;Cyclopentane,

1,1,2,3,3,4,5-heptafluoro-, stereoisomer;Cyclopentane, 1,1,2,3,3,4,5-heptafluoro-,

stereoisomer;Cyclopentane, 1,1,2,3,3,4,5-heptafluoro-, cis,cis-;Cyclopentene,

1,3,3,4,5,5-hexafluoro-;Cyclopentene, 1,2,3,3,4,5-hexafluoro-, cis-;Cyclopentane,

1,1,2,3,4,5-hexafluoro-, stereoisomer;Cyclopentane, 1,1,2,3,4,5-hexafluoro-,

(2.alpha.,3.alpha.,4.beta.,5.alpha.)-;Cyclopentane, 1,1,2,3,4,5-hexafluoro-,

stereoisomer;Cyclopentene, 1,3,4,4,5,5-hexafluoro-;Cyclopentene,

3,3,4,4,5,5-hexafluoro-;Cyclopentene, 1,2,3,4,5-pentafluoro-;Cyclopentene,

1,3,4,5,5-pentafluoro-;Cyclopentane, 1,1,2,2,3,3,4,5-octafluoro-;Cyclopentane,

1,1,2,2,3,4,4,5-octafluoro-;Cyclopentane, 1,1,2,3,4,5-hexafuoro-;Cyclopropane,

2-ethenyl-1,1-difluoro-;Cyclopropane, 1,1-difluoro-2,3-dimethyl-, trans-;Cyclopropane, 1,

1-difluoro-2, 3-dimethyl-, cis-;Cyclobutane, 1,1,2,2-tetrafluoro-3-methylene-;Cyclobutane,

1,1,2,2,3,4-hexafluoro-3-(trifluoromethyl)-;Cyclopentane, nonafluoro-;Cyclobutane,

1,1,2,2-tetrafluoro-3-methyl-;Cyclopropane, 1,2-bis (trifluoromethyl)-;Cyclobutene,

1,3,3,4,4-pentafluoro-2-methyl-;Cyclopropane, 1,1-difluoro-2,3-dimethyl-;Cyclopropane,

1-methyl-1-(trifluoromethyl)-;Cyclopropane, 1,1-difluoro-2,2-dimethyl-;1-Butyne,

1,3,4,4,4-pentafluoro-3-(trifluoromethyl)-;1,3-Pentadiene,

1,1,2,3,4,5,5,5-octafluoro-;Cyclobutene,

1,2,3,3,4-pentafluoro-4-(trifluoromethyl)-;1,3-Pentadiene,

1,1,2,3,4,5,5,5-octafluoro-;Spiro(2.2)pentane, octafluoro-;Pentadiene,

octafluoro-;1,2-Butadiene, 1,1,4,4,4-pentafluoro-3-(trifluoromethyl)-;1,2-Pentadiene,

1,1,3,4,4,5,5,5-octafluoro-;Cyclopropane, pentafluoro (trifluorovinyl)-;1,3-Pentadiene,

1,1,2,3,4,5,5,5-octafluoro-;1,4-Pentadiene, 1,1,2,3,3,4,5,5,-octafluoro-;Cyclopropene,

3,3-difluoro-1,2-bis(trifluoromethyl)-;Cyclopentene, octafluoro-;1,3-Butadiene,

1,1,2,4,4-pentafluoro-3-(trifluoromethyl)-;Cyclobutene,

1,3,3,4,4-pentafluoro-2-(trifluoromethyl)-;2-Pentyne, 1,1,1,4,4,5,5,5-octafluoro-;2-Pentene,

1,1,1,2,3,4,4,5,5,5-decafluoro-;1-Butene,

1,1,3,3,4,4,4-heptafluoro-2-(trifluoromethyl)-;Cyclopropane,

1,1,2,3-tetrafluoro-2,3-bis(tribluoromethyl)-, cis-;Cyclopropane,

1,1,2,3-tetrafluoro-2,3-bis(trifluoromethyl)-, trans-;2-Pentene,

1,1,1,2,3,4,4,5,5,5-decafluoro-;Cyclopropane, pentafluoro(pentafluoroethyl)-;Cyclopropane,

1,1,2,3-tetrafluoro-2,3-bis(trifluoromethyl)-;Cyclopropane,

1,1,2,2-tetrafluoro-3,3-bis(trifluoromethyl)-;Cyclopentane, decafluoro-, radical ion

(1-);2-Pentene, 1,1,1,2,3,4,4,5,5,5-decafluoro-;2-Butene,

1,1,1,2,4,4,4-heptafluoro-3-(trifluoromethyl)-;Pentylidene,

1,2,2,3,3,4,4,5,5,5-decafluoro-;1-Butene,

1,1,2,3,4,4,4-heptafluoro-3-(trifluoromethyl)-;Pentene, decafluoro-;Cyclobutane,

heptafluoro(trifluoromethyl)-;1-Pentene, 1,1,2,3,3,4,4,5,5,5-decafluoro-;Cyclopentane,

decafluoro-;2-Cyclobuten-1-one, 2,3,4,4-tetrafluoro-;Furan, tetrafluoro-;Silane,

tetrakis(trifluoromethyl)-;Silane, trifluoro(nonafluorobutyl)-;Pentane,

1,1,1,2,2,4,5,5,5-nonafluoro-;Pentane, 1,1,1,2,2,3,5,5,5-nonafluoro-;Pentane,

1,1,1,2,2,3,3,4,5-nonafluoro-;Pentane, 1,1,1,2,3,3,5,5,5-nonafluoro-;Propane,

1,1,1,3,3,3-hexafluoro-2-methyl-2-(trifluoromethyl)-;Butane,

1,1,1,2,4,4-hexafluoro-2-(trifluoromethyl)-;Pentane, 1,1,2,2,3,3,4,4,5-nonafluoro-;Butane,

1,1,1,4,4,4-hexafluoro-2-(trifluoromethyl)-;Propane,

1,1,1,3,3,3-hexafluoro-2,2-dimethyl-;Pentane, 1,1,3,3,5,5-hexafluoro-;Butane,

1,1,1,2,3,3-hexafluoro-2-methyl-;Pentane, hexafluoro-;Pentane, 1,2,3,3,4,5-hexafluoro-;Butane,

2-(difluoromethyl)-1,1,1,2-tetrafluoro-;Butane,

1,1,1-trifluoro-2-(trifluoromethyl)-;Butane-1-13C, 4,4,4-trifluoro-3-(trifluoromethyl)-;Pentane,

1,1,1,5,5,5-hexafluoro-;Pentane, 1,1,1,2,3,3-hexafluoro-;Pentane, 2,2,3-trifluoro-;Pentane,

2,2,4-trifluoro-;Butane, 1,1,1-trifluoro-2-methyl-;Butane, 1,1,1-trifluoro-2-methyl-;Butane,

1,2,2-trifluoro-3-methyl-;Butane, 1,3,3-trifluoro-2-methyl-;Butane,

2,2,3-trifluoro-3-methyl-;Butane, 1,1,1-trifluoro-2-methyl-;Butane,

1,1,2-trifluoro-3-methyl-;Pentane, 1,1,2-trifluoro-;Propane,

1,1,1-trifluoro-2,2-dimethyl-;Pentane, 1,1,1-trifluoro-;Butane, 1,1,1-trifluoro-3-methyl-;Silane,

(nonafluorobutyl)-;Silane, dimethylbis (trifluoromethyl)-;Silane, (difluoromethyl)

(fluoromethyl)methyl (trifluoromethyl)-;Silane, bis(difluoromethyl)bis(fluoromethyl)-;Silane,

(3,3,3-trifluoro-2-(trifluoromethyl)propyl)-;Silane, trimethyl(trifluoromethyl)-;Silane,

trifluoro(1-methylpropyl);Silane, (difluoromethyl) (fluoromethyl) dimethyl-;Silane,

tris(fluoromethyl)methyl-;Silane, (1,1-dimethylethyl)trifluoro-;Silane,

trifluoro(2-methylpropyl)-;Silane, methyl(3,3,3-trifluoropropy)-;Silane, butyltrifluoro-;

EXAMPLE 51

In a preferred embodiment, the dispersed phase
can be composed of any chemical which has a boiling
point under standard pressure conditions below the
body temperature of the organism to which the
formulation is to be administered and which will be
examined following administration by ultrasound.
Example 45 discusses how one selects suitable
chemicals for the dispersed phase based on the

temperature range obtained by consideration of the boiling point of the selected chemical and parameters of the manufacturing process.

Fluorocarbons, because of their low toxicity, good emulsification properties, and low water solubility, leading to persistent microbubbles, are especially suitable as chemicals from which to select the dispersed phase:

1,2,2-tris (trifluoromethyl) propane • 2,2-bis (trifluoromethyl) propane • 2-methyl-2 trifluoromethyl propane • tetrakis (trifluoromethyl) silane • methyl tris (trifluoromethyl) silane • bis (trifluoromethyl) dimethyl silane • trifluoromethyl trimethyl silane   1,1-bis (trifluoromethyl) -2,2,3,3-tetrafluoro cyclopropane • 1,1-bis (trifluoromethyl) cyclopropane • 1,1-bis (trifluoromethyl) 2,2 difluoro cyclopropane • 1,1-dimethyl (-2,2,3,3)-tetrafluoro cyclopropane • 2,2 difluoro 1-methyl-1-trifluoromethyl cyclopropane • 1,2-bis (trifluoromethyl) -1,2,3,3 tetrafluoro cyclopropane (cis+trans) • 1,2-bis (trifluoromethyl) -1,2-difluoro cyclopropane (ccs + trans) • 1,2-bis (trifluoromethyl) -3,3 difluoro cyclopropane • 1,2-bis (trifluoromethyl) cyclopropane (ccs + trans) 1,1,2,2,4,4,5,5-octafluoro spiro [2.2] pentane • 1,1,2,2,-tetrafluoro spiro [2.2] pentane • 1,1,4,4-tetrafluoro spiro [2.2] pentane • 1,1,5,5-tetrafluoro spiro [2.2] pentane • 3,3,4,5 tetrafluoro furar • tris (tri fluoromethyl) phosphire • 1,1,2,2,3,3,4,4,5,5, decafluoro cyclopentane   1,2,2,3,4,4,5,5-octafluoro bicyclo [1.1.1] pentane • 2,2,4,4,5,5 hexafluoro bicyclo [1.1.1] pentane • 1,2,2,3,4,4-hexafluoro bicyclo [1.1.1] pentane • 1,2,2,3-tetrafluoro bicyclo [1.1.1] pentane 2,2,3,3-tetrafluoro bicyclo [1.1.1] pentane • 1,2,2,3,3,4,4-pentafluoro -1-trifluoromethyl cyclobutane, 2,2,3,4,4-pentafluoro -1-trifluoromethyl bicyclo [1.1.0] butane, 2,2,4,4-tetrafluoro 1-trifluoromethyl bicyclo [1.1.0] butane • bicyclo [2.1.0] pentane.

EXAMPLE 52

The following emulsions were formulated and tested according to methods described in Example 18.

5   All solutions were made as a 2% solution in saline. A volume of 0.1 cc of each chemical was comminuted with 5 cc saline through a 3-way stopcock for 25 passes. A volume of 1.0 mL of the mixture was immediately injected through a 1.2 μm filter into a stirring water bath containing 1000 mL water at 37°C.

10   The resulting backscatter was then recorded with the use of a Hewlett-Packard 77020A Ultrasound System at 5.0 mHz.

Chemical	B.P. (°C)	Vapor Pressure	M.W.	Ratio of Persistence	Ratio of Intensity
saline + air				1	0.0
Nonane	151	10 mmHg at 20°C	128.3	9	0.5
1,2, Dichloroethane	83	87 mmHg at 25°C	98.9	6	0.25
Halothane	50	300 mmHg at 25°C	197.4	6	0.25
Perfluorodecalin	141	6.6 mmHg at 25°C	462.1	9	2.0
Dodecafluoropentane	29	646 mmHg at 25°C	288.1	24	5.0

The chemical with the lowest boiling point and highest vapor pressure, dodecafluoropentane, produced the most backscatter (brightest contrast) which persisted the longest and slowly diminished over 4-5

25   minutes. The high boiling and low vapor pressure chemicals, nonane and perfluorodecalin, resulted in some backscatter (less pronounced than dodecafluoropentane) which rapidly diminished within 1.5 minutes with perfluorodecalin providing greater

30   backscatter than nonane. The ethanes, dichloroethane and halothane, also resulted in minimal backscatter that diminished to baseline within 1 minute. Mixture of saline and air provided the least amount of backscatter which persisted for 5-10 seconds.

35   If the degree of persistance of saline+air is set as 1, then dodecafluoropentane would be 24-times greater. If backscatter intensity is qualitatively ranked from 0 to 5, then saline+air would be 0 and

dodecafluoropentane would be 5 with nonane, 1,2,-
dechloroethane, halothane and perfluorodecaline being
0.5, 0.25, 0.25 and 2.0, respectively.

EXAMPLE 53

5   The objective of this study was to evaluate the
potential that an intravenous administration of the
emulsions of the invention, at doses effective in
producing ultrasound contrast, to New Zealand White
rabbits would produce the hyperinflated non-
10   collapsible lung (HNCL) syndrome. HNCL syndrome has
been produced by a number of fluorocarbon emulsions,
including 20% Fluosol®, an F.D.A.-approved
intravascular perfluorochemical emulsion (described
in patent JP 1609986 and incorporated herein by
15   reference), emulsions containing
perfluorooctylbromide (described in patent US4987154
and incorporated herein by reference), and other
fluorocarbon emulsions (described in patents or
applications EP 231091, JP 63060943, US 4859363, US
20   5171755, and JP 21196730, incorporated herein by
reference). The mechanism of HNCL syndrome
production, its potential reversibility, and the
clinical significance are not known. The syndrome is
characterized by lungs which are hyperinflated at
25   necropsy, have an increased total volume, a decreased
mean density, and contain detectable quantities of
the administered fluorocarbon in the tissues. Leland
Clark, the discoverer of HNCL, has stated (Clark LC,
et al., Biomat., Art. Cells & Immob. Biotech., 20,
30   1085-1099, 1992, incorporated herein by reference)
that "if HNCL occurs in other species (i.e., humans),
then only fluorocarbons boiling above 150°C can be
considered safe."
     Four groups of male New Zealand White rabbits
35   (3 per group) were intravenously administered the

emulsion of Example 44 at 0.2 or 1.0 mL/kg
bodyweight, Fluosol (Alpha Therapeutic Corp.) at
24 mL/kg bodyweight, or saline at 24 mL/kg. The
doses were selected based on a dose which produces

5   ultrasound contrast. Body weights, food consumption,
and clinical observations were made during and
immediately following administration. Twenty-four
hours after administration the rabbits were
euthanized, the lungs excised, and the degree of

10   inflation graded, the weights and volumes of the
lungs measured, and the presence of perfluorocarbons
in the tissue determined by gas chromatography, using
a head space analyzer.
        The lungs of rabbits receiving saline, or the

15   emulsion of Example 44 were normal at necropsy,
collapsing upon opening the thorax. The lungs of the
rabbits receiving Fluosol showed moderate to severe
inflation.
        There were no treatment-related changes among

20   the groups in lung weights or lung-weight-to-
bodyweight ratio. The lung volume, lung-volume-to-
bodyweight ratio, and lung density measurements in
the rabbits administered the emulsion of Example 44
were unchanged compared to controls. The

25   administration of Fluosol lead to a 175% increase in
lung volume, a 185% increase in lung-to-body weight
ratio, and a 45% decrease in lung density when
compared to controls. These changes were highly
significant (p=0.001).

30   Dodecafluoropentane was not detected during
analysis of lung tissue from any animal in the group
receiving the emulsions of Example 44. Fluosol
contains four major peaks and one minor peak by gas
chromatographic analysis. All five peaks were found

35   in gas chromatograms of headspace tissue samples from
animals receiving Fluosol.

Under the conditions of the study, a single
administration of the emulsion of Example 44 at
dosages producing excellent ultrasound contrast
showed no effect on lung inflation, weight, or
density, did not yield detectable levels of
dodecafluoropentane in lung tissues, and is not
considered to cause the hyperinflated non-collapsible
lung syndrome in the rabbit.

The emulsions formed by the methods described in
the prior art produced this unsafe condition at doses
which were necessary to produce ultrasound contrast,
while surprisingly, emulsions with fluorocarbons
which boil as low as 29°C, formulated by the methods
described in the instant application, did not produce
HNCL.

EXAMPLE 54.

A pharmacokinetic study was performed in beagle
dogs administered a single intravenous dose of the
emulsion of Example 44 over 5-8 seconds at 0.05,
0.10, and 0.15 mL/kg by obtaining multiple, timed
blood samples and quantifying the dodecafluoropentane
content by a validated gas chromatography assay.
Twenty-four dogs, twelve males and twelve females,
were studied in three dosage groups.

The data was fitted to a two compartment model
with a bolus input and a first order output. There
was no significant difference when comparing the
males and females separately or when comparing the
three dosage groups.

The distribution phase varied from 0.9 to
1.3 minutes. The elimination phase varied from 30 to
48 minutes. The t_{max} (time to maximum concentration
in the second compartment) varied from 5.1 to
6.6 minutes. These elimination times are compared to
the elimination times of fluorocarbon emulsions of

the prior art which are measured in months (see Clark
et al. above). Clearly an imaging agent which clears
the body in a matter of hours is preferred.

EXAMPLE 55

Emulsions of dodecafluoropentane (boiling point
28-29°C), a mixture of dodecafluoropentane and
decafluorobutane with a boiling point of 20.0°C, and
perfluorocyclopentane (boiling point of 22.5°C) were
formed and their echogenicity tested. The emulsions
contained Fluorad 170 C as surfactant and were formed
by applying acoustic energy from a waterbath
sonicator. Echogenicity was tested by adding 0.2 mL
of each emulsion to 1000 mL of water at 37°C through
a 1.2 micron filter and measuring the videodensity by
the methods described in Example 1. The emulsion
containing dodecafluoropentane produced a grayscale
intensity six seconds following administration of
58.5 units (background of 2.9), the mixture of
fluorocarbons produced an increase of 3.0 to 133.3
under the same conditions, and the
perfluorocyclopentane produced the greatest increase,
of from 3.6 to 158.9. Thus, the lower boiling
fluorocarbons produced greater echogenicity than the
higher boiling fluorocarbons.

EXAMPLE 56

Useful ultrasound contrast agent formulations
are formed by stabilizing dispersions of a low
boiling chemical with emulsions containing a
dispersed phase which is composed of chemicals which
themselves do not vaporize to an appreciable extent
at the body temperature of an organism undergoing an
ultrasound examination. For example, fluorocarbon-
or hydrocarbon-containing emulsions which are
composed of high boiling dispersed phases, as

described in US 4,767,410, US 4,987,154, JP 2196730,
JP 1609986, JP 63060943, and EP 245019, incorporated
herein by reference can form the basis of a
formulation in which the backscatter efficiency is

5   greatly enhanced by the addition of a high vapor
pressure chemical. For example, lecithin-stabilized
perfluorooctylbromide emulsions have significantly
increased echogenicity if perfluorocyclopentane
(boiling point = 22°C) is added to the dispersed

10   phase prior to comminution. Other low boiling
organic halides, hydrocarbons, or ethers have the
same effect.

Although the invention has been described in
some respects with reference to specified preferred

15   embodiments thereof, many variations and
modifications will be apparent to those skilled in
the art. It is, therefore, the intention that the
following claims not be given a restrictive
interpretation but should be viewed to encompass such

20   variations and modifications that may be routinely
derived from the inventive subject matter disclosed.

What is claimed is:

1.   A stable, biocompatible colloidal
dispersion comprising a liquid dispersed phase and an
aqueous continuous phase, said dispersed phase
5   comprising a liquid having a boiling point below
about 40°C.

2.   The colloidal dispersion of claim 1
wherein said chemical is selected from the group
consisting of aliphatic hydrocarbons, organic
10   halides, and ethers, where each member of the group
has six or fewer carbon atoms.

3.   The colloidal dispersion of claim 1 wherein
said chemical is a fluorine-containing compound.

4.   The colloidal dispersion of claim 1 wherein
15   said fluorine-containing compound has a molecular
weight of less than 300.

5.   The colloidal dispersion of claim 2 wherein
said chemical is selected from the group consisting
of n-pentane, isopentane, neopentane, cyclopentane,
20   butane, cyclobutane, decafluorobutane,
dodecafluoropentane, dodecafluoroneopentane,
perfluorocyclopentane.

6.   The colloidal dispersion of claim 1 wherein
said dispersion further comprises an amphiphilic
25   material.

7.   The colloidal dispersion of claim 6 wherein
said amphiphilic material has a relative HLB matched
to said dispersed phase liquid.

8.. The colloidal dispersion of claim 6 wherein
said amphiphilic material comprises a biocompatible
protein.

9. The colloidal dispersion of claim 6 wherein
5   said amphiphilic material comprises at least one
surfactant.

10. The colloidal dispersion of claim 8 wherein
said protein is selected from the group consisting of
albumin, fibrinogen, fibrin, serum globulins,
10   hemoglobin, myoglobin, and immunoglobulins.

11. The colloidal dispersion of claim 6 wherein
said amphiphilic material comprises a
polyoxypropylene-polyoxyethylene glycol nonionic
block copolymer.

15   12. The colloidal dispersion of claim 11
wherein said amphiphilic material is selected from
the group consisting of Poloxamer 181, 188, 231, 282,
331, 401, 402, and 403.

13. The colloidal dispersion of claim 7 wherein
20   said amphiphilic material comprises a fluorine-
containing surfactant.

14. The colloidal dispersion of claim 13
wherein said fluorine containing surfactant is
selected from the group consisting of Zonyl FSO, FSN,
25   FSA, and FSJ.

15. The colloidal dispersion of claim 7 wherein
said amphiphilic material is selected from the group
of surfactants consisting of anionic, cationic,
30   nonionic, or zwitterionic molecules.

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16. The colloidal dispersion of claim 7 wherein
said amphiphilic material comprises at least one
surfactant selected from the group of surfactants
which contain, as hydrophilic groups, one or more of
5   the following chemical groups: sulfonate, sulfate,
carboxylate, phosphate, ammonium, quaternary
ammonium, betaines, sulfobetaines, polyoxyethylene,
polyols, alcohols, ethers, polypeptide, or
polyglycidyl; and as hydrophobic groups, one or more
10   of the following chemical groups: fatty acids,
paraffins, olefins, alkyl benzenes, alcohols,
alkylphenols, polyoxypropylenes, polypeptides,
fluorocarbons, and silicones.

17. The colloidal dispersion of claim 7 wherein
15   said amphiphilic material is present at a
concentration such that the interfacial tension
between water and the liquid dispersed phase is less
than 26 dynes/cm.

18. The colloidal dispersion of claim 7 wherein
20   said amphiphilic material is present at a
concentration greater than 0.001% by weight per
volume.

19. The colloidal dispersion of claim 1 wherein
said dispersion further comprises a viscogen.

25   20. The colloidal dispersion of claim 19
wherein said viscogen is selected from the group
consisting of glucose, iohexol, iopamidol, iopentol,
sorbitol, sucrose, and polyethylene glycol.

21. The colloidal dispersion of claim 19
30   wherein the viscogen is present at a concentration

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sufficient to produce a viscosity greater than 1.1
cP.

22. The colloidal dispersion of claim 19
wherein the viscogen is present at a concentration of
5   between 0.001 and 75% by weight per volume.

23. The colloidal dispersion of claim 1 wherein
said liquid dispersed phase comprises particles
having an average diameter less than 1000 nm.

24. The colloidal dispersion of claim 1 wherein
10   the concentration of said liquid dispersed phase is
between 0.00001 to 166% by weight per volume.

25. The colloidal dispersion of claim 1 wherein
the aqueous medium comprises an additive selected
from the group of acidifying agents, alkalizing
15   agents, antimicrobial preservatives, antioxidants,
buffering agents, chelating agents, complexing
agents, solubilizing agents, humectants, solvents,
suspending agents, viscosity-increasing agents and
tonicity agents.

20   26. The colloidal dispersion of claim 25
wherein said additive is present at a concentration
such that the osmolarity of said aqueous medium is at
least 250 mOm.

27. The colloidal dispersion of claim 1 wherein
25   said liquid comprises a chemical selected from the
group of chemicals containing 4 to 17 atoms.

28. The colloidal dispersion of claim 1 wherein
said liquid comprises a chemical selected from the
group of chemicals of the form $C_5H_xF_y$.

29. A biocompatible colloidal dispersion for use in ultrasound imaging of an animal having a body temperature T comprising a dispersed phase and an aqueous continuous phase, said dispersed phase

5   including a chemical with a sufficiently high vapor pressure that a portion of said chemical is a gas at the temperature T.

30. A method of ultrasound imaging in an animal comprising the steps of:

10   (1) preparing a stable, biocompatible colloidal dispersion comprising a liquid dispersed phase and an aqueous continuous phase, said dispersed phase comprising a liquid having a boiling point below the body temperature of said animal.

15   (2) administering said dispersion to said animal to be imaged and waiting a time sufficient for said liquid dispersed phase to form microbubbles; and

(3) performing an ultrasound scan on a portion of said animal in which said microbubbles are

20   distributed.

31. A method as in claim 30 wherein said chemical is selected from the group consisting of aliphatic hydrocarbons, organic halides, and ethers having six or fewer carbon atoms.

25   32. A method of preparing a storage stable colloidal dispersion comprising the steps of

(a) mixing at least one amphiphilic material with water to form an aqueous continuous phase;

(b) adding a liquid having a boiling point of

30   less than 37°C to said continuous phase;

(c) comminuting the mixture manually, mechanically, or by the action of ultrasound for a

time sufficient to form a dispersion of particles
with an average diameter of less than 5000 nm.

        33.   A method of preparing a storage stable
colloidal dispersion comprising the steps of

5   (a) mixing at least one amphiphilic material
with water to form an aqueous continuous phase;
           (b) adding an amount of a gas which has a
boiling point less than 37°C to said continuous
phase; and

10   (c) condensing said gas to form a liquid
dispersed phase of particles with an average diameter
of less than 5000 nm.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5   A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAOCS, J. AM. OIL CHEM. SOC., VOL. 66, NO. 10, PAGE(S) 1515-23, 1989 Zarif, Leila et al 'Synergistic stabilization of perfluorocarbon-Pluronic F-68 emulsion by perfluoroalkylated polyhydroxylated surfactants' see the whole document	1-29
X	RADIOLOGY, DEC. 1982, VOL. 145, NO. 3, PAGE(S) 759-62, Mattrey, R.F. et al 'Perfluoroctylbromide: a liver/spleen-specific and tumor-imaging ultrasound contrast material.' see the whole document	1-29

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| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 1994	0 1. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Dullaart, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. FLUORINE CHEM., VOL. 50, NO. 3, PAGE(S) 271-84, 19 August 1990 KABALNOV, A. S. ET AL 'Solubility of fluorocarbons in water as a key parameter determining fluorocarbon emulsion stability' see page 275 - page 276 see "RESULTS AND DISCUSSION"	1-29
X	WO,A,92 15284 (MICRO VESICULAR SYSTEMS, INC.) 17 September 1992 see claims; examples	1-29
X	WO,A,89 06978 (SCHERING A.G.) 10 August 1989 see the whole document	1-29
X	WO,A,91 12823 (DELTA BIOTECHNOLOGY LTD.) 5 September 1991 see the whole document	1-29
P,X	DE,A,41 27 442 (ZENTRALINSTITUT FÜR ANORGANISCHE CHEMIE) 18 February 1993 see the whole document	1-29
P,X	EP,A,0 554 213 (SINTETICA SA (SYNT-) SYNTHETICA SA) 4 August 1993 see claims; examples	1-29
P,X	WO,A,93 05819 (S.C.QUAY) 1 April 1993 see claims; examples	1-29
Y	EP,A,0 077 752 (SCHERING A.G.) 27 April 1983 see claims; examples	6-18
Y	WO,A,92 11873 (BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH) 23 July 1992 see claims; examples	6-18

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:   1-31
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In view of the large number of compounds, which are defined by the general definitions according to claims 1-4,6-9,13,15-19,21-27,29-31, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given, and to the general idea UNDERLYING THE APPLICATION (SEE GUIDELINES, PART B, CHAPTER III, PARAGRAPH 3.6).

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of item 2 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9215284	17-09-92	US-A-	5219538	15-06-93
		AU-A-	1538892	06-10-92
		EP-A-	0573592	15-12-93
WO-A-8906978	10-08-89	DE-C-	3803971	07-09-89
		DE-A-	3803972	10-08-89
		AU-B-	635200	18-03-93
		AU-A-	3035189	25-08-89
		EP-A-	0327490	09-08-89
		EP-A-	0398935	28-11-90
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		DE-D-	4290068	07-10-93
		EP-A-	0494615	15-07-92
		EP-A-	0566601	27-10-93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9211873		HU-A- NZ-A-	64239 241246	28-12-93 26-01-94